Dysfunctional high-density lipoproteins in coronary heart disease: implications for diagnostics and therapy

Annema, Wijtske ; von Eckardstein, Arnold

Abstract: Low plasma levels of high-density lipoprotein (HDL) cholesterol are associated with increased risks of coronary heart disease. HDL mediates cholesterol efflux from macrophages for reverse transport to the liver and elicits many anti-inflammatory and anti-oxidative activities which are potentially anti-atherogenic. Nevertheless, HDL has not been successfully targeted by drugs for prevention or treatment of cardiovascular diseases. One potential reason is the targeting of HDL cholesterol which does not capture the structural and functional complexity of HDL particles. Hundreds of lipid species and dozens of proteins as well as several microRNAs have been identified in HDL. This physiological heterogeneity is further increased in pathologic conditions due to additional quantitative and qualitative molecular changes of HDL components which have been associated with both loss of physiological function and gain of pathologic dysfunction. This structural and functional complexity of HDL has prevented clear assignments of molecules to the functions of normal HDL and dysfunctions of pathologic HDL. Systematic analyses of structure-function relationships of HDL-associated molecules and their modifications are needed to test the different components and functions of HDL for their relative contribution in the pathogenesis of atherosclerosis. The derived biomarkers and targets may eventually help to exploit HDL for treatment and diagnostics of cardiovascular diseases.

DOI: https://doi.org/10.1016/j.trsl.2016.02.008

Dysfunctional high-density lipoproteins in coronary heart disease: implications for diagnostics and therapy

Wijtske Annema[1], Arnold von Eckardstein[1]

[1]*Institute of Clinical Chemistry, University Hospital Zurich, Zurich, Switzerland*

Corresponding author: Arnold von Eckardstein, Institute of Clinical Chemistry, University Hospital Zurich, Rämistrasse 100, CH-8091 Zurich, Schwitzerland, tel. +41 (0)44 2255 22 60, email address: arnold.voneckardstein@usz.ch

Running head: Dysfunctional HDL

Abbreviations: ABCA1 = ATP-binding cassette transporter A1; ACS = acute coronary syndrome; ADCY9 = adenylate cyclase 9; apo = apolipoprotein; CETP = cholesteryl ester transfer protein; CAD = coronary artery disease; CHD = coronary heart disease; CKD = chronic kidney disease; eNOS = endothelial nitric oxide synthase; GPCRs = G-protein-coupled receptors; HDL = high-density lipoproteins; HOCl = hypochlorous acid; ICAM-1 = intracellular cell adhesion molecule 1; IL = interleukin; IVUS = intravascular ultrasound; LCAT = lecithin:cholesterol acyltransferase; LDL = low-density lipoproteins; LOX-1 = lectin-like oxidized LDL receptor-1; MDA = malonaldehyde; miRNA = microRNA; MMP-9 = matrix metalloproteinase 9; PON1 = paraoxonase 1; PAPC = 1-palmitoyl-2-arachidonyl-*sn*-glycero-3-phosphocholine; PLPC = 1-palmitoyl-2-linoleoyl-*sn*-glycero-3-phosphocholine; POPC = 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine; PPAR-α = peroxisome proliferator activator receptor alpha; rHDL = reconstituted HDL; S1P = sphingosine-1-phosphate; SAA = serum amyloid A; SDMA = symmetric dimethylarginine; SR-BI = scavenger receptor class B type I;

STEMI = ST-elevation myocardial infarction; TLR = toll-like receptor; TNF-α= tumor necrosis factor α; VCAM-1 = vascular cell adhesion molecule 1.

Abstract

Low plasma levels of high-density lipoprotein (HDL) cholesterol are associated with increased risks of coronary heart disease (CHD). HDL mediates cholesterol efflux from macrophages for reverse transport to the liver and elicits many anti-inflammatory and anti-oxidative activities which are potentially anti-atherogenic. Nevertheless, HDL has not been successfully targeted by drugs for prevention or treatment of cardiovascular diseases. One potential reason is the targeting of HDL cholesterol which does not capture the structural and functional complexity of HDL particles. Hundreds of lipid species and dozens of proteins as well as several microRNAs have been identified in HDL. This physiological heterogeneity is further increased in pathological conditions due to additional quantitative and qualitative molecular changes of HDL components which have been associated with both loss of physiological function and gain of pathological dysfunction. This structural and functional complexity of HDL has prevented clear assignments of molecules to the functions of normal HDL and dysfunctions of pathological HDL. Systematic analyses of structure-function-relationships of HDL-associated molecules and their modifications are needed to test the different components and functions of HDL for their relative contribution in the pathogenesis of atherosclerosis. The derived biomarkers and targets may eventually help to exploit HDL for treatment and diagnostics of cardiovascular diseases.

Introduction

According to epidemiological, pathophysiological, genetic, and clinical evidence dyslipidemias play an important pathogenic role in the development of atherosclerosis. Notably, low-density lipoprotein (LDL)-cholesterol lowering by the use of statins has become one of the most successful developments in preventive medicine since they help to reduce coronary heart disease (CHD) event rates by up to 50% in the highest dosage.[1] Besides intensified LDL-cholesterol lowering, increasing of high-density lipoprotein (HDL)-cholesterol has been another interesting target for cardiovascular risk reduction for a long time. Many clinical and epidemiological studies as well as meta-analyses thereof have shown the inverse relationship of HDL-cholesterol plasma levels with the risk of CHD.[2] Furthermore, the development of atherosclerotic lesions could be decreased or even reverted in several animal models by transgenic over-expression or exogenous application of apolipoprotein A-I (apoA-I), that is the most abundant protein of HDL.[3]

For LDL-cholesterol and high blood pressure this type of epidemiological and biological evidence has been successfully translated into drugs that lower CHD risk. To date, however, it has been proven difficult to successfully reduce CHD risk with drugs increasing HDL-cholesterol such as fibrates, niacin, or inhibitors of cholesteryl ester transfer protein (CETP).[4, 5] Moreover, in several inborn errors of human HDL metabolism and genetic mouse models with altered HDL metabolism, the changes in HDL-cholesterol levels were not associated with the opposite changes in cardiovascular risk and atherosclerotic plaque load, respectively, expected from epidemiological studies.[3, 6] Because of these controversial data, the causal role and hence suitability as a therapeutic target of HDL has been increasingly questioned.

However, previous intervention and genetic studies targeted LDL-cholesterol and HDL-cholesterol, that is the cholesterol measured by clinical laboratories in LDL and HDL, respectively. By contrast to the pro-atherogenic and hence disease causing cholesterol in LDL (that is LDL-cholesterol), which after internalization turns macrophages of the arterial intima

into pro-inflammatory foam cells, the cholesterol in HDL (that is HDL-cholesterol) neither exerts nor reflects any of the potentially anti-atherogenic activities of HDL. By contrast to LDL-cholesterol, HDL-cholesterol is only a non-functional surrogate marker for estimating HDL particle number and size without deciphering the heterogeneous composition and hence functionality of HDL.[5] In a prototypic HDL particle two to five molecules of apoA-I and about 100 molecules of phosphatidylcholine form an amphipathic shell in which several molecules of un-esterified cholesterol are imbedded and which surrounds a core of completely water-insoluble cholesterol esters, albeit less, triglycerides. Already molar differences in the content of the major protein and lipid constituents of HDL, that is apoA-I, phosphatidylcholine, sphingomyelin, cholesterol, and cholesteryl esters, cause considerable heterogeneity of HDL in shape, size, and charge.[5] Some of these model particles have been artificially reconstituted for experimental but also therapeutic purposes.[7] This macro-heterogeneity is further increased by the presence or absence of quantitatively minor proteins or lipids, some of which may contribute to the pleiotropic functions of HDL. Previous proteomic and lipidomic studies revealed a much greater structural complexity: HDL particles carry more than eighty different proteins and hundreds of lipid species.[8] Most recently, even microRNAs (miRNAs) were found to be transported by HDL.[9, 10] Many of these molecules are not passive cargo but biologically active and contribute to the pleiotropic and potentially anti-atherogenic properties of HDL. This micro-heterogeneity is further increased in HDL of patients with various inflammatory diseases, including CHD, by the loss or structural modification of typical HDL constituents or by the acquisition of atypical constituents.[5, 11, 12] Of note, many physiological as well as pathological components and modifications are present at concentrations which are several orders of magnitude lower than the concentration of HDL-cholesterol or even HDL particles and hence reflected by measurements of neither HDL-cholesterol, nor apoA-I, nor HDL-particle concentrations.[5] In the search for biomarkers that reflect the functionality of HDL

better than these high-throughput markers, bioassays have been developed for clinical studies as well as for discovery of functional biomarkers by proteomics and lipidomics.

HDL and protection against cardiovascular disease

HDL particles exert many beneficial actions that may help protect against cardiovascular disease.[5, 13]

The classical anti-antherogenic role of HDL in cardiovascular disease is its potential to drive cholesterol export from macrophage foam cells and subsequent transport towards the liver for excretion into bile and feces, i.e. reverse cholesterol transport.[14, 15] HDL elicits the first step, cholesterol efflux from macrophages, by several mechanisms and different subclasses. The lipid-containing, buoyant, and α-migrating HDL facilitates aqueous diffusion of plasma membrane residing cholesterol by tethering to scavenger receptor class B type I (SR-BI). α-migrating HDL also elicits active cholesterol efflux from intracellular pools by a mechanism involving ATP-binding cassette transporter G1 (ABCG1). Although present at very low concentrations lipid-free apoA-I, which because of its electrophoretic mobility is termed prebeta1-HDL, is a very potent inducer of active cholesterol efflux by ABCA1.[4, 5, 13-15] Both ABCA1 and ABCG1 are integrated into both positive feed-forward and negative feed-back regulation loops of cellular cholesterol homeostasis involving both transcriptional regulation by oxysterol-activated nuclear liver X receptors and post-transcriptional regulation by several miRNAs. Thus, cholesterol efflux is determined by the extracellular concentration and composition of HDL particles as well as by the activity of ABC transporters.[4, 5, 13-15] By modulating cellular cholesterol homeostasis, both apoA-I and HDL indirectly regulate survival and functions of several cell types (Figure 1).[4, 5]

Numerous other potential atheroprotective functions of HDL have been described and the list is still growing. An important event in the development of atherosclerosis is the modification of LDL, notably the formation of oxidized LDL. Many *in vitro* experiments have indicated that

HDL, primarily by its associated components apoA-I and paraoxonase 1 (PON1), has the ability to counteract lipid peroxidation of LDL.[16-19] Furthermore, HDL functions as a protective factor for the vascular endothelium by mechanisms that involve interactions with signaling receptors and hence go beyond the regulation of cholesterol homoeostasis or the inhibition of oxidation (Figure 1). HDL induces endothelium-dependent vascular relaxation via increased endothelial nitric oxide synthase (eNOS) phosphorylation and nitric oxide production,[20] and maintains endothelial barrier stability by promoting endothelial cell survival,[21, 22] stimulating endothelial junction closure via HDL-bound sphingosine-1-phosphate (S1P),[23, 24] and accelerating endothelial cell migration and re-endothelialization of injured arteries.[25, 26] Moreover, anti-inflammatory activities of HDL in atherosclerosis have been documented. HDL attenuates the adhesion of monocytes to the vascular endothelial lining and subsequent monocyte transendothelial migration by decreasing the expression of adhesion molecules on endothelial cells[27, 28] and monocytes[29] as well as by suppressing the expression of chemokines and chemokine receptors.[30, 31] Eventual disruption of atherosclerotic lesions initiates platelet activation and aggregation. In the presence of HDL platelet activation and aggregation are significantly inhibited,[32-34] which may in turn lead to reduced thrombus formation.[32] Other proposed mechanisms by which HDL may reduce the risk of atherothrombosis are increased activities of the anticoagulants activated protein C and protein S,[35] reduced synthesis of platelet-activing factor and tissue factor by endothelial cells,[36, 37] and neutralization of procoagulatory anionic phospholipids.[38]

Dysfunctional HDL in cardiovascular disease

Although HDL initially has emerged as a particle with potent atheroprotective properties, more recent work demonstrated that HDL is rendered dysfunctional in individuals with cardiovascular disease and even may become pro-atherogenic. Already in 2000, Navab et al. postulated that atherosclerotic cardiovascular disease is associated with the conversion of HDL

from anti-oxidative into dysfunctional pro-oxidative particles.[16] The authors revealed that HDL particles isolated from coronary artery disease (CAD) patients fail to inhibit oxidation of LDL by human arterial wall cells, are unable to prevent the chemotactic effect of LDL on monocytes, and do not reduce the ability of LDL-derived oxidize phospholipids to stimulate monocyte adherence to endothelial cells, whereas HDL from healthy control subjects show beneficial effects.[16] Since then, a broad spectrum of dysfunctions of HDL in patients with cardiovascular diseases has been described, including a reduced cholesterol efflux capacity from macrophages and other cells, impaired anti-oxidative effects, a reduced ability to inhibit adhesion molecule expression on endothelial cells, a lack of the normal ability to stimulate endothelial nitric oxide bioavailability as well as diminished activities to promote endothelial cell survival.[5, 39] There are several examples of dysfunction where HDL was not only characterized by loss or reduction of normal functionality but by the gain of atypical functions. For example, HDL of patients with CAD or chronic kidney disease (CKD) was found to inhibit rather than stimulate nitric oxide production because it gained the ability to interact with the lectin-like oxidized LDL receptor LOX-1 and the toll-like receptors TLR2 and TLR4, respectively.[40, 41] Dysfunctional HDL in cardiovascular disease is not limited to CAD and acute coronary syndrome (ACS). Also in other cardiovascular diseases, such as heart failure,[42] ischaemic cardiomyopathy,[43] heart transplantion,[44, 45] as well as in many conditions that are known to increase cardiovascular risk such as diabetes, CKD, acute and chronic inflammatory diseases, or familial hypercholesterolemia,[46-48] HDL has been found to lose its atheroprotective characteristics. Since we have previously reviewed the modifications by disease state,[5] we here focus on alterations of HDL found in patients with CAD.

Cholesterol efflux capacity as a risk factor for cardiovascular disease

Several studies investigated the association of present or incident CAD with the capacity of apoB-depleted serum or plasma –a surrogate of HDL– to elicit efflux of labeled cholesterol

from macrophages. This activity is widely called cholesterol efflux capacity. Various protocols have been described that differ by the cell type used and by the use of pharmacological agents to investigate specific cholesterol efflux pathways, that is ABCA1-, ABCG1- and/or SR-BI-mediated cholesterol efflux.

A pioneering case-control study found that the capacity of apoB-free serum to elicit cholesterol efflux from cyclic AMP activated J774 macrophpages is inversely related to carotid intima media thickness and associated with decreased odds of having CAD independent of HDL-cholesterol levels.[49] Two case-control cohort studies supported these earlier observations regarding the inverse association between cholesterol efflux capacity and prevalent CAD.[50]

Several studies have addressed the usefulness of cholesterol efflux capacity as a predictor for cardiovascular disease events and got inconsistent findings. The first prospective analysis on HDL function reported by Li et al. unexpectedly showed a higher cell-derived cholesterol efflux capacity being associated with a higher rather than a lower incident risk for nonfatal myocardial infarction/stroke and major adverse cardiovascular events, i.e. nonfatal myocardial infarction, nonfatal stroke, or death.[50] In contrast, Rohatgi et al, revealed in a biobank of 2924 participants from the multiethnic Dallas Heart Study that the risk for future atherosclerotic cardiovascular events (nonfatal myocardial infarction, nonfatal stroke, coronary revascularization, or death from cardiovascular causes) decreases with increasing quartiles of cholesterol efflux capacity.[51] In 2450 probands of the LURIC study who underwent diagnostic coronary angiography, cholesterol efflux capacity was also inversely associated with cardiovascular mortality during 10 years of follow-up.[52] In addition, in a nested case-control study from the prospective EPIC-Norfolk study cholesterol efflux capacity was found to be an independent risk factor for the development of fatal or nonfatal CHD.[53] However, in renal transplant recipients, baseline cholesterol efflux capacity was not associated with future cardiovascular mortality or all-cause mortality.[54] Of note, in this study low baseline cholesterol efflux capacity was identified as an

independent predictor of renal graft failure, a condition, which usually occurs due to progressive atherosclerosis in the vasculature of the transplanted kidney.[54]

Strikingly, all published prospective studies on HDL function thus far used different methods to measure cholesterol efflux capacity. Li et al. used the murine macrophage RAW 264.7 cells and cholesterol efflux assays in participants of the Dallas Heart Study, the EPIC-Norfolk Study, and the LURIC study were performed with mouse J774 macrophages, whereas in the renal transplant recipients study cholesterol efflux capacity was quantified by measuring the efflux of labeled cholesterol from human THP-1 macrophages. Because of the stimulation by cyclic AMP, the studies on J774 cells and RAW 264.7 cells mainly recorded ABCA1-mediated cholesterol efflux, whereas the study on THP1 cells recorded all cholesterol efflux pathways. Although the Dallas Heart Study and the EPIC-Norfolk Study both used J774 macrophages and although both demonstrated that cholesterol efflux capacity is associated with incident cardiovascular disease,[51, 53] cholesterol efflux capacity measured with use of fluorescence-labeled cholesterol (applied in the Dallas Heart Study) only poorly correlated ($r = 0.54$) with measurements performed with radiolabeled cholesterol (applied in the LURIC and EPIC-Norfolk Studies).[51] Vice versa, the capacity of serum to elicit cholesterol efflux from RAW 264.7 or J774 cells correlated rather strongly ($r = 0.92$), yet Li et al. did not find any association of cholesterol efflux capacity with incident CHD.[50] Because of the conflicting results and methodological differences, the causal contribution of HDL dysfunction to cardiovascular diseases is as yet not proven. The proof will depend on the elaboration of the molecular basis of disturbed cholesterol efflux capacity.

In this context it must also be noted that in the large studies apoB-free serum or plasma rather than isolated HDL was incubated with cells for several hours. During this rather long incubation time bioactive molecules of the serum or plasma may enter the cell and modify the activity of the cellular cholesterol efflux machinery. Notably, the cell surface abundance and activity of the cholesterol efflux pump ABCA1 is known to be regulated by free fatty acids, cytokines, and

drugs. The cholesterol efflux capacity may hence not only record HDL functionality but also adverse regulatory effects of other plasma components on the cells.

Finally, cholesterol efflux capacity of HDL cannot be taken as a general surrogate of HDL functionality because HDL exerts many other functions that are independent of cholesterol efflux. These other functions, for example towards endothelial cells, are difficult to be measured in large cohort studies.[4, 11, 12] Therefore, it is important to unravel the molecular basis of HDL dysfunction. The structural correlates of HDL dysfunction, whether HDL-subclasses, proteins or lipids or modifications thereof, can be much more easily translated into robust high throughput analytical methods than the recording of HDL functionality by cell-based bioassays.

Compositional changes associated with dysfunctional HDLs in cardiovascular disease

Cardiovascular disease and HDL dysfunction have been associated with changes in the protein or lipid composition of HDL (Table 1).

Serum amyloid A

HDL particles from patients with ACS are enriched in proteins involved in the acute-phase response, such as serum amyloid A (SAA) und complement C3.[55, 56] SAA is a common acute-phase protein synthesized by the liver in response to inflammatory cytokines. During an inflammatory response, excessive SAA can displace apoA-I from the HDL surface and in extreme circumstances SAA can account for up to 80% of the HDL proteins.[56, 57] The contribution of HDL-bound SAA to HDL dysfunctionality remains a matter of debate. Cell culture experiments indicated that the ability of HDL to remove cholesterol from human macrophages is only significantly attenuated, when SAA constitutes at least 50% of total protein in HDL,[58] a situation that is rarely achieved in patients with cardiovascular diseases. Other studies have identified SAA as an effective acceptor for cellular cholesterol efflux via SR-BI.[59] Furthermore, SAA-containing acute phase HDL or HDL isolated from mice overexpressing

SAA through adenoviral gene transfer efficiently acquired cholesterol from fibroblasts through the ABCA1-dependent pathway.[59] In sharp contrast, it has been found that acute-phase rabbit HDL containing approximately 30% SAA stimulated less cholesterol efflux from J774 macrophages than native HDL.[60] Moreover, in mice, acute inflammation induced by silver nitrate impaired the capacity of HDL to promote cholesterol efflux *ex vivo*.[61] This effect was not observed in mice lacking SAA1 and SAA2,[61] suggesting that SAA1/2 was responsible for the decreased HDL cholesterol efflux activities in mice undergoing inflammatory stimulation with silver nitrate. Notably, adenoviral overexpression of human SAA1 in mice diminished net *in vivo* reverse cholesterol transport from macrophages to feces.[62] One of the mechanisms that might contribute to decreased reverse cholesterol transport in response to SAA overexpression is the inhibitory effect of SAA on SR-BI-mediated selective uptake of cholesteryl esters from HDL.[63] Other important functions of HDL that may be influenced by the SAA content are its anti-inflammatory, vasoprotective, and anti-oxidative effects. Enrichment of HDL with SAA reduced its ability to inhibit thrombin-induced monocyte chemotactic protein-1 expression in vascular smooth muscle cells, and in patients with end stage renal disease, the anti-inflammatory function of HDL correlated inversely with the accumulation of SAA in the particle.[64] Moreover, it has been demonstrated that native HDL particles supplemented with SAA are no longer functional in inhibiting tumor necrosis factor α (TNF-α)-induced expression of vascular cell adhesion molecule 1 (VCAM-1) on endothelial cells and subsequent adhesion of mononuclear cells.[65] However, this was not confirmed by others using artificial reconstituted HDL (rHDL).[66] Further experiments indicated that SAA-modified HDL lacks the capacity to promote endothelial production of nitric oxide.[65] Also, SAA-rich HDL loses most of its PON1 and platelet activating factor acetylhydrolase activity,[67] two enzymes that account for the anti-oxidative potential of HDL. Finally, SAA has been shown to facilitate the retention of HDL by vascular proteoglycans,[68] which increases its susceptibility to oxidative and enzymatic modifications that may render it dysfunctional.

Despite the numerous studies on the influence of SAA on HDL function, it is as yet unclear whether the presence of SAA in HDL can lead to an increased risk of cardiovascular disease. This issue has been partially addressed in the Ludwigshafen Risk and Cardiovascular Health Study, a prospective cohort of 3310 patients referred for coronary angiography.[65] In this study, higher HDL-cholesterol levels were paradoxically associated with an increased risk of all-cause and cardiovascular mortality in patients with SAA concentrations above the 80th percentile,[65] implying that the protective effects of HDL are lost in patients with very high circulating SAA levels.

Apolipoprotein C-III

There is some evidence for a potential role of apoC-III in relation to HDL dysfunction in cardiovascular disease. The abundance of apoC-III in HDL particles is increased in subjects with CAD and ACS.[21, 69-71] Moreover, HDL isolated from patients, who experienced a first myocardial infarction before the age of 35, contained elevated levels of apoC-III.[72] An analysis of data from the Nurses' Health study and the Health Professionals Follow-Up Study revealed an inverse risk association of cholesterol measured in HDL free of apoC-III, but a positive risk association with cholesterol measured in apoC-III-containing HDL.[73] In this regard it is noteworthy that rather prevalent nonsense mutations in the APOC3 gene cause a decrease in apoC-III plasma concentrations and triglycerides as well as an increase in HDL-cholesterol. Concomitantly, the carriers had an increased chance of CHD-free survival, which was greater than expected from the changes in lipoprotein concentrations.[74, 75] Some missense mutations in APOC3 encode for structural apoC-III mutants, which show reduced abundance in HDL and cause a similar lipoprotein phenotype as the nonsense mutations.[76] It may hence be that the genetically determined lack of apoC-III in HDL improves the functionality and anti-atherogenicity of HDL.

In vitro experiments showed that the presence of apoC-III on HDL impeded its ability to inhibit adhesion of THP-1 monocytes to endothelial cells.[77] Further proof for a putative involvement of apoC-III in HDL dysfunction in cardiovascular disease came from a study demonstrating that HDL from CAD and ACS patients are defective in protecting endothelial cells from apoptosis.[21] The anti-apoptotic capacity of HDL from CAD patients could be restored by a blocking antibody for apoC-III, while HDL from healthy subjects no longer suppressed apoptosis of endothelial cells after supplementation with apoC-III.[21]

Triglycerides

HDL dysfunction in patients with cardiovascular disease, especially in the setting of hypertriglyceridemia, may also be driven by an increase in the HDL triglyceride content. The high CETP activity in hypertriglyceridemic states favors the net transfer of cholesteryl esters in HDL to apoB-containing lipoproteins in exchange for triglycerides, thereby generating relatively triglyceride-enriched HDL particles.[78] This corroborates with data showing that the HDL content of triglycerides is elevated in patients with extensive coronary artery stenosis,[79] in patients after acute myocardial infarction,[56] and in patients who had undergone coronary artery bypass grafting 34-38 hours previously.[80] Concomitant with triglyceride enrichment, the level of cholesteryl esters within HDL decreases.[79, 80] The effects of triglyceride accumulation in HDL on cholesterol efflux are controversial. Some reported an enhanced cholesterol efflux capacity in hypertriglyceridemic patients as well as in hyperlipidemic patients in the postprandial state, both exhibiting an abnormally high triglyceride content of HDL.[81-83] On the other hand, it has been demonstrated that the HDL_3 subfraction obtained from patients with familial hypercholesterolemia was enriched in triglycerides but had a reduced ability to mobilize labeled-cholesterol from cultured human macrophages.[47] Surprisingly, although cellular cholesterol efflux mediated by isolated HDL was reduced in statin-treated coronary patients, there was no difference between normolipidemic patients and dyslipidemic patients

with low HDL-cholesterol and high triglycerides.[84] Of additional interest for the reverse cholesterol transport pathway, increased concentrations of triglycerides within HDL were shown to interfere with SR-BI-mediated selective uptake of HDL-derived cholesteryl esters in hepatocytes.[83, 85] Additional studies conducted in patients with familial hypercholesterolemia provided evidence that patient HDL enriched in triglycerides is less effective than healthy control HDL in inhibiting the release of interleukin-8 (IL-8) from cultured endothelial cells.[47] Nevertheless, it should be noted that all published data so far on the association between HDL triglyceride content and HDL dysfunction were obtained using HDL isolated from subjects with disease conditions. Hence, the associations between HDL triglycerides and HDL dysfunction may simply confound other functionally more relevant changes in the HDL proteome or lipidome. Experiments showing the influence of supplementation of HDL with triglycerides on HDL functional characteristics are required to definitely establish whether the amount of triglycerides within HDL has functional relevance.

Glycerophospholipids

Lipidomic approaches have identified different phospholipid subclasses on the surface of HDL including, -in order of decreasing abundance- phosphatidylcholines, sphingomyelins, lysophosphatidylcholines, phosphatidylethanolamines, phosphatidylinositols, plasmologens, phosphatidylserines, phosphatidylglycerols, phosphatidic acid, and cardiolipins.[78, 86] Two independent cross-sectional studies in male patients undergoing coronary angiographic examination have reported negative associations between serum levels of HDL phospholipids and the extent of coronary occlusion.[87, 88] Case-control studies applying lipidomics revealed statistically significant differences in the composition of phospholipid species between CAD patients, ACS patients, and healthy subjects.[89, 90]

Variations in the phospholipid composition of the HDL surface lipid monolayer are major determinants of the size, net surface charge, and rigidity of HDL particles, all of which may

affect their anti-atherosclerotic actions. Particularly, it has been proposed that the physical properties of the HDL phospholipid monolayer play a key role in the anti-oxidative function of HDL.[91] Decreased HDL surface rigidity facilitated the transfer of lipid hydroperoxides from LDL to HDL and hence the subsequent inactivation by redox-active Met residues of apoA-I.[91] In fact, most of the phosholipid molecular species identified by liquid-chromatography-mass spectrometry/mass spectrometry (LC-MS/MS) profiling of HDL particles could be linked to an atheroprotective biological activity of HDL.[86] Especially, a strong relationship existed between the content of negatively charged phosphatidylserine and various metrics of HDL function, i.e. the cholesterol efflux capacity from THP-1 cells, the anti-oxidative activity towards LDL, the ability to counteract platelet activation, the anti-inflammatory properties in a cell-free assay, and the potential to protect endothelial cells from apoptosis.[86]

Artificial enrichment of HDL_3 with phosphatidylcholines increased SR-BI-mediated cholesterol efflux.[92] The same investigators subsequently showed that enhanced *in vivo* hydrolysis of HDL phospholipids by overexpression of endothelial lipase in human apoA-I transgenic mice caused pronounced reductions in the serum phospholipid/apoA-I ratio and attenuated the capacity of serum to mediate cholesterol efflux via SR-BI by 90%, whereas the ABCA1-dependent efflux potential increased by 63%.[93] The opposite was observed in mice injected with an adenovirus expressing a phosphatidylserine-specific phospholipase.[93] Moreover, the cholesterol efflux capacity of rHDL differed with the phospholipid acyl chain length and the degree of unsaturation.[94] Also, the magnitude of inhibition of VCAM-1 expression in activated human umbilical vein endothelial cells varied markedly between discoidal rHDL composed of different phospholipid species.[95] At equivalent apoA-I molarity, rHDL containing 1-palmitoyl-2-linoleoyl-*sn*-glycero-3-phosphocholine (PLPC) or 1-palmitoyl-2-arachidonyl-*sn*-glycero-3-phosphocholine (PAPC) repressed TNF-α-induced VCAM-1 expression in endothelial cells by 95% and 70%, respectively, whereas rHDL

containing 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC) achieved only a 16% inhibition.[95]

Indirect evidence for an association between HDL dysfunction, HDL phospholipid content, and cardiovascular disease, was derived from the results of a clinical study showing that HDL isolated from CAD patients with very high HDL-cholesterol levels contained less phospholipids and had a lower macrophage cholesterol efflux potential as compared to HDL isolated from healthy subjects with very high HDL-cholesterol levels.[96] Somewhat contradictory, the increased content of lysophosphatidylcholine and phosphatidic acid in HDL from patients with an ST-elevation myocardial infarction (STEMI) has been associated with diminished cholesterol efflux and anti-oxidative activities.[90]

Plasmalogens are a class of glycerophospholipids with anti-oxidative properties that are present in HDL only in low amounts.[78, 97-99] Patients with CAD had HDL relatively depleted of the plasmalogen species PC33:3, PC35:2 and PC34:2 and with a compromised ability to inhibit apoptosis of endothelial cells.[89] The finding that incorporation of the plasmalogen PC35:2 in rHDL improved its anti-apoptotic activity towards endothelial cells further underscored the potential importance of plasmalogens for the anti-apoptotic activity of HDL.[89]

Sphingolipids

Sphingolipids are represented in HDL by sphingomyelins, ceramides, lysosphingolipids, glycosphingolipids, gangliosides, and sulfatides.[78]

In animal studies the infusion of rHDL containing the apoA-I mimetic 5A, POPC, and sphingomyelin induced more pronounced mobilization of tissue cholesterol and more profound plaque regression than sphingomyelin-free control rHDL.[100] rHDL containing sphingomyelin was also found to be a more potent inhibitor of cytokine release.[100] In line with this observation, the presence of coronary heart disease in post-menopausal women was inversely related to sphingomyelin in HDL.[101]

The lysosphingolipid S1P is probably the most intensively studied sphingolipid of HDL. S1P binds to HDL via its specific binding-protein apoM[102] and interacts with five different G-protein coupled S1P receptors expressed by various cells of the cardiovascular system. HDL also elicits efflux of S1P from erythrocytes by apoM-dependent and –independent mechanisms.[103] Although present on only one of 10 to 20 HDL particles, S1P contributes to several of the protective functions of HDL. For example, HDL-associated S1P appears to be important for the vasoprotective effects on endothelial cell survival,[104, 105] endothelial migration,[105] endothelial adhesion molecule expression,[106, 107] endothelial barrier integrity,[23, 108] endothelial tube formation,[109] and nitric oxide-dependent vasorelaxation.[20] It also inhibits vascular smooth muscle cell migration and chemokine production,[31, 110] cardiomyocyte apoptosis during hypoxia-reoxygenation,[111, 112] and myocardial damage after ischemia/reperfusion.[112, 113] Of note, at least some functions of S1P appear to be only exerted if S1P is present on HDL.[107] This is of note because only about 50% of S1P in plasma is transported by HDL. The rest is transported by albumin (about 30%) and apoB-containing lipoproteins.[102, 114]

Quantification of S1P in apoB-free plasmas of patients with monogenic disorders of HDL metabolism have illustrated that very high HDL-cholesterol levels do not automatically translate into a higher abundance of S1P in HDL,[114] suggesting that measurements of the S1P level within HDL may provide additional information beyond plasma levels of HDL-cholesterol or apoA-I. In fact, initial observational studies suggest that the S1P content of HDL is a potential biomarker of cardiovascular disease. One study reported 4 to 5 times higher amounts of S1P in HDL of healthy control subjects compared to HDL particles of patients with stable CAD.[115, 116] Another case-control study also found S1P concentrations lowered in HDL of patients with ACS or CAD compared to control HDL, but much less pronounced.[89] Moreover, the concentration of HDL-bound plasma S1P was inversely proportional to the severity of angina symptoms in CAD patients.[115] In agreement with these data, in patients presenting with stable CAD, HDL-bound plasma S1P levels decreased depending on the

number of vessels affected by the disease.[117] In a nested case control study involving 204 subjects stratified by HDL-cholesterol from the Copenhagen City Heart Study, S1P levels in the HDL-containing fraction of serum (depleted of apoB-containing lipoproteins) were inversely associated with the occurrence of ischemic heart disease.[108] Although a cross-sectional analysis in patients with CAD showed that patients who possessed HDL-S1P levels in the lowest quartile were more likely to demonstrate in-stent restenosis than patients in the higher quartiles,[118] the amount of HDL-associated S1P was not predictive for the development of restenosis during follow-up in CAD patients after percutaneous coronary intervention.[117]

Additional findings of studies on HDL isolated from acute myocardial infarction patients suggest that a reduced S1P content of HDL impairs the capacities to stimulate endothelial nitric oxide production[119] and to inhibit endothelial apoptosis.[89] Moreover, a reduced HDL-associated S1P content has been related to defects in S1P-dependent activation of ERK1/2 and Akt signaling pathways as well as eNOS phosphorylation at Ser1177 in vascular endothelial cells in patients with CAD.[116] *In vitro* loading of both healthy and CAD HDL particles with additional S1P improved the effects of HDL on endothelial ERK1/2 and Akt activation, eNOS-activating phosphorylation, and vasorelaxation in pre-contracted arteries.[116] This loading may not only be an experimental model but may also happen *in vivo*, because HDL induces S1P efflux from erythrocytes and other cells by apoM dependent and –independent mechanisms.[103] It may hence well be that HDL are locally uploaded with S1P by endothelial cells or circulating blood cells so that the *ex vivo* measured S1P levels in HDL underestimate the *in vivo* situation.

microRNAs

HDL was also found to contain miRNAs. *In vitro* experiments showed that HDL delivers miRNAs to hamster baby kidney cells and human hepatocytes through SR-BI.[9] HDL derived from patients with familial hypercholesterolemia or ACS exhibited a miRNA signature pattern distinct from that of normal control HDL.[9, 10] These findings raised the hypothesis that the

miRNA cargo carried by HDL may influence its functionality. The content of miR-223 was found increased in HDL isolated from familial hypercholesterolemia subjects.[9, 10] Interestingly, miR-223 has been described to be a post-transcriptional regulator of various aspects of cholesterol metabolism; miR-223 represses the SR-BI-mediated selective uptake of cholesteryl esters from HDL and cholesterol biosynthesis and by increasing ABCA1 expression promotes cholesterol efflux towards apoA-I.[120] HDL was shown to deliver miR-223 into endothelial cells, where it downregulates expression of intracellular cell adhesion molecule 1 (ICAM-1).[121] However, these findings are in contrast to those by Wagner et al.[10] who confirmed the presence of low copy numbers of miRNAs in HDL, but found no evidence that HDL delivers physiologically relevant amounts of miRNAs into endothelial cells, smooth muscle cells, and peripheral blood mononuclear cells.[10] In general, it is important to emphasize the low concentration of miRNAs in HDL, which is beyond particle concentration. Many HDL particles would need to interact with a single cell to deliver enough miRNA molecules that are sufficient for RNA interference. If at all this will more likely happen by autocrine or paracrine regulation of neighboring cells rather than by endocrine regulation of distant organs. Further investigation is necessary to prove or disprove that HDL-bound miRNAs are of functional importance and play a role in the development of cardiovascular disease.

Modifications of HDL components and anti-apoA-I auto-antibodies

Under inflammatory and oxidative conditions lipids and proteins of HDL undergo certain molecular modifications, which directly compromise their beneficial biological properties and can produce immunogenic neo-epitopes which trigger the generation of auto-antibodies which interfere with HDL function.

Myeloperoxidase-mediated protein modifications

Myeloperoxidase (MPO) is a heme enzyme released by neutrophil granulocytes and other myeloid cells that can cause protein modifications in HDL (Table 2). In the presence of chloride ions and hydrogen peroxide MPO generates the chlorinating intermediate hypochlorous acid (HOCl), which can further react with nitrite to generate nitrylchloride.[122] Alternatively, MPO combines with hydrogen peroxide to convert nitrite into nitric dioxide radicals, which are potent nitrating intermediates.[122] Previous studies have already introduced MPO as a risk factor for cardiovascular diseases. Plasma levels of MPO are elevated early in the course of myocardial infarction.[123, 124] Moreover, in patients who presented to the emergency department with chest pain, circulating MPO levels at presentation predicted acute myocardial infarction as well as the need of revascularization, myocardial infarction, and major adverse coronary events in the subsequent 30 days and 6 months.[123, 124] The preferred target for MPO-catalyzed oxidation in HDL is apoA-I. The levels of two MPO-specific oxidation products, 3-nitrotyrosine and 3-chlorotyrosine, are abundant within apoA-I or HDL particles recovered from either plasma of patients with established cardiovascular disease or human atherosclerotic lesions.[125-127] In apoA-I of circulating HDL, the favored modification site for MPO is tyrosine residue Tyr-192.[128] ApoA-I with nitrated or chlorinated residues Tyr-192, Tyr-18, and Tyr-166 or oxidized Trp-72 has also been isolated from human atheroma tissues.[128-131] Immunohistochemical stainings showed that macrophages in the human atherosclerotic intima are immunoreactive for MPO and that the location of immunoreactivities for 3-nitrotyrosine and HOCl-modified proteins are closely associated with the presence of apoA-I.[126, 127] Moreover, the relative proportion of modified apoA-I is much larger in the atherosclerotic plaque than in plasma.[128-130] Taken together, these observations suggest that MPO oxidizes apoA-I in the atherosclerotic intima rather than in the circulation.

Oxidation of apoA-I and HDL with the MPO/H_2O_2/Cl^- system compromised their capacity to induce ABCA1-dependent cholesterol efflux.[125, 129, 132, 133] Similar experiments using pre-incubation of apoA-I or HDL with HOCl yielded identical results.[127, 132] The marked defect in

apoA-I-promoted cholesterol efflux via the ABCA1 pathway following oxidation with MPO has been ascribed by some authors to a combination of chlorination of Tyr-192 and methionine oxidation.[134, 135] In line with this proposal, the extent of chlorination of Tyr-192 and sulfoxidation of Met-148 were higher in apoA-I of subjects with stable CAD and ACS and inversely correlated with the capacity of serum HDL to remove cellular cholesterol through ABCA1.[135] Also, apoA-I with oxidative modification of the tryptophan residue Trp-72, a form of apoA-I that is highly prevalent in apoA-I recovered from atheroma and increased in the plasma of patients with cardiovascular disease or CAD, had significantly lower cholesterol acceptor capacity.[131] Mechanistically, MPO-dependent chlorination of apoA-I interfered with the binding of apoA-I to ABCA1 and as a consequence chlorinated apoA-I failed to activate the accompanying JAK2 signaling.[132] However, it must be noted that in plasma less than 200 of 1 million apoA-I molecules contain oxidized Tyr-192 or Trp-72 residues.[129, 131] In view of their very low quantity it is unlikely that these modifications reduce cholesterol efflux capacity of (apoB-free) plasma or HDL by simple loss-of-function in the interaction with ABCA1. Rather additional gain of dysfunction is necessary to explain reduced ABCA1-mediated cholesterol efflux capacity of plasma by these modifications. The situation is different for sulfoxidized Met-148, which is found in up to 30% of apoA-I molecules in plasma. However, in atherosclerotic plaques 20% of apoA-I was found to contain oxidized Tyr-192 or Trp-72 residues,[129, 131] so that these modifications may be of local pathogenic relevance independently of their impact on cholesterol efflux capacity of plasma.

Oxidative modification of Met-148 or nitration of Tyr-166 by MPO in apoA-I was also associated with a loss of apoA-I-mediated activation of lecithin:cholesterol acyltransferase (LCAT),[130, 133, 136] which is crucial to help maintain a concentration gradient for cholesterol efflux from the cell to the HDL particle surface. The injection of purified MPO into mice reduced the net movement of labeled-cholesterol from macrophages to plasma and feces.[62] Fisher's group demonstrated that the infusion of non-oxidized apoA-I but not apoA-I oxidized

by MPO facilitates reverse cholesterol transport from macrophages in apoA-I knockout mice and beneficially alters plaque composition in hyperlipidemic apoE knock-out mice.[137]

Besides functional consequences for cellular cholesterol efflux and reverse cholesterol transport, exposure of human HDL to the MPO/H_2O_2/Cl^- system has also been found to ablate the ability of HDL to suppress serum starvation-induced capase-3 activation and thereby apoptosis in endothelial cells and to promote eNOS activation.[133] Furthermore, HDL modified by MPO enhanced rather than inhibited the protein expression of VCAM-1 on endothelial cells treated with TNF-α.[133] The loss of anti-apoptotic and anti-inflammatory functions of HDL following MPO treatment has been explained by the inability of MPO-oxidized HDL to bind to SR-BI.[133] However, our lab did not find any reduced binding of HDL from CAD patients with endothelial dysfunction.[40] As discussed before, the very small proportion of apoA-I molecules containing MPO-modified amino acid residues is also unlikely to explain the reduced endothelial functionality of HDL from CAD patients by loss-of-function in SR-BI interaction. By contrast gain-of-dysfunction, for example the atypical interaction with LOX-1,[40] can explain how quantitatively very minor modifications can lead to endothelial dysfunction.

Protein carbamylation

Another posttranslational modification of HDL-associated proteins that may actively contribute to HDL dysfunction is protein carbamylation. Carbamylation of proteins results from the nonenzymatic reaction between isocyanic acid and the ε-amino group of lysine residues or the N-terminus of proteins.[138] In humans, isocyanic acid is a product of the spontaneous decomposition of urea, but at sites of inflammation and atherosclerotic plaques it may also derive from the oxidation of thiocyanate by MPO.[138, 139] Double immunofluorescence assays revealed colocalization of MPO with carbamylated proteins within atherosclerotic plaques.[139] Intriguingly, HDL isolated from human atherosclerotic lesions carried high amounts of carbamyllysines.[140, 141] These results are consistent with the assumption that HDL trapped in

the vascular wall becomes a target for carbamylation. *In vitro* carbamylation by the MPO/thiocyanate/H_2O_2 system or cyanate has been shown to transform HDL into pro-atherosclerotic particles. Exposure of aortic endothelial cells to carbamylated HDL induced apoptosis of these cells and carbamylated HDL was clearly less potent at preventing endothelial apoptosis elicited by growth factor withdrawal.[139] Also, HDL particles modified through carbamylation not only had poorer cholesterol efflux activities, but instead facilitated further accumulation of cholesterol in human monocytes.[140] Another important observation is that cyanate-treated HDL had lower LCAT activity.[141] Additional studies demonstrated that carbamalytion reduced the activity of PON1 within HDL and hence the concomitant anti-oxidative effects of HDL against LDL oxidation.[141]

Symmetric dimethylarginine

Symmetric dimethylarginine (SDMA) is the structural isomer of the eNOS inhibitor asymmetric dimethylarginine and the concentration of circulating SDMA is an independent predictor for cardiovascular mortality in the general population[142] as well as in patients with CHD.[143] The HDL-SDMA content may have important functional implications with respect to the vascular effects of HDL. In patients with CKD, SDMA has been found within HDL particles and linked to the functional impairment of HDL in these patients.[41] HDL from patients with CKD and HDL enriched with SDMA inhibited rather than increased endothelial nitric oxide production, endothelial cell migration, and endothelial repair as well as stimulated superoxide release by endothelial cells, and when injected in mice, raised arterial blood pressure.[41] The adverse effects of SDMA within HDL on endothelial cells are due to the interaction of SDMA with TLR2 on endothelial cells.[41] Stimulation of TLR2 by HDL-associated SDMA initiates signaling cascades leading to diminished Akt-dependent eNOS-activating phosphorylation at Ser473 and increased eNOS-inhibiting phosphorylation at Thr495 as well as activation of NADPH oxidase and thereby superoxide production.[41] However, these changes were only

reported for patients with CKD. As yet, no data are available on the relationship between HDL-bound SDMA and cardiovascular diseases and their association with HDL dysfunction.

Paraoxonase-1 and lipid peroxidation products

Several laboratories suggested the inactivation of HDL-associated PON1 as a cause of HDL dysfunction in cardiovascular disease. PON1 is an antioxidant enzyme that is carried mostly on HDL particles and the positive effects of HDL on LDL oxidation are related to the ability of PON1 to hydrolyze lipid peroxides. Additionally, low PON1 activity in HDL may increase the susceptibility of HDL to oxidation. The elevated oxidative stress burden in cardiovascular diseases may result in the formation of lipid peroxidation products in HDL, which in turn may negatively affect their beneficial features. In fact, HDL from mice lacking PON1 were unable to prevent the accumulation of lipid peroxides in LDL and these mice demonstrated an increased susceptibility to atherosclerosis,[144] while introduction of the human PON1 transgene in mice had opposite effects on the anti-oxidative functions of HDL and atherosclerosis.[145] Interestingly, if residing on the same HDL particle, PON1 and MPO were found to interact and reciprocally affect each other's enzymatic activity.[146] PON1 inhibited MPO peroxidase activity, whereas in parallel site-specific oxidative modification of PON1 by MPO impaired PON1 function.[146]

Recent studies linked the loss of endothelial HDL functionality in CAD and ACS patients to the increased content of the lipid peroxidation product malondialdehyde (MDA) in HDL due to reduced PON1 activity.[40] HDL-mediated nitric oxide production in endothelial cells was found impaired in patients with either stable CAD or ACS.[40] This impairment was the result of decreased eNOS-activating phosphorylation at Ser1177 and eNOS-inhibiting phosphorylation at Thr495 as well as increased endothelial superoxide production in response to patient HDL.[40] Likewise, HDL from patients with stable CAD and ACS did not inhibit cytokine-induced adhesion molecule expression on endothelial cells, induced adhesion of monocytes to

endothelial cells under basal conditions, and failed to promote endothelial regrowth after arterial injury.[40] The adverse effects of HDL from CAD patients on endothelial nitric oxide production could be improved by blocking either LOX-1 or protein kinase C βII.[40] Modification of HDL from healthy control subjects with MDA compromised their ability to induce endothelial nitric oxide production via mechanisms involving the activation of LOX-1 and protein kinase C βII.[40] In addition, heart failure has been associated with the generation of dysfunctional pro-inflammatory HDL particles, which increased endothelial release of chemotactic activity for monocytes and exhibited reduced PON1 activity.[42] The accumulation of oxidation products of arachidonic and linoleic acids, presumably attributable to the inactivation of PON1, in HDL from patients with heart failure was discussed to contribute to the change in functionality.[42] There are also data indicating that the concentration of lipid peroxidation products is higher in HDL from type 2 diabetic patients with cardiovascular disease than in patients with type 2 diabetes but no known cardiovascular disease.[147]

Anti-apoA-I autoantibodies

Modifications of apoA-I can give rise to immunogenic neo-epitopes and thus lead to the appearance of autoantibodies to apoA-I. Increased levels of anti-apoA-I antibodies were initially described in patients with autoimmune diseases, such as systemic lupus erythematosus and antiphospholipid syndrome.[148] A few years later, Vuilleumier et al. found that 11-21% of ACS patients tested positive for anti-apoA-I autoantibodies compared with 20% of patients with severe internal carotid stenosis, 38% of non-STEMI patients, 29% of patients undergoing elective carotid endarterectomy, and 1-2% of healthy controls.[149-152] In fact, there was an association between high anti-apoA-I IgG titers and increased circulating levels of oxidized LDL, TNF-α, IL-6, IL-8, and matrix metalloproteinase 9 (MMP-9).[150, 153, 154] Accordingly, patients positive for serum anti-apoA-I IgG had decreased plaque stability, as evidenced by increased intraplaque MMP-9 levels as well as higher densities of macrophages and neutrophils

within plaques.[151] These clinical findings are corroborated by *in vivo* and *in vitro* data showing that apoE-deficient mice immunized with anti-apoA-I IgG developed larger, more vulnerable atherosclerotic lesions with higher neutrophil and MMP-9 contents and reduced collagen content, displayed signs of myocardial injury, and had higher mortality rates than apoE-deficient mice treated with a control IgG.[151, 155] Furthermore, incubation of human monocyte-derived macrophages with anti-apoA-I IgG under cell culture conditions resulted in increased production of cytokines (TNF-α, IL-6, IL-8), matrix metalloproteinases (MMP-9), and monocyte chemoattractants (CCL2, CXCL8).[151, 153, 154] Blocking experiments revealed that anti-apoA-I IgG-induced inflammatory responses in macrophages are mediated mainly via the CD14/TLR2 complex and to a lesser extent via TLR4.[154] Consistent with a role for TLR2 and TLR4 in the pro-atherosclerotic effects of anti-apoA-I antibodies, the augmented atherosclerotic plaque vulnerability, myocardial necrosis, and mortality in response to anti-apoA-I IgG passive immunization are abrogated in TLR2- and TLR-4-deficient mice.[155] Moreover, anti-apoA-I autoantibodies exerted a positive chronotropic effect on cultured rat cardiomyocytes, potentially explaining the higher resting heart rate observed in ACS patients positive for anti-apoA-I IgG.[156] Longitudinal analyses established anti-apoA-I IgG positivity as an independent predictor of major adverse cardiovascular outcomes in patients with rheumatoid arthritis[153] as well as one year after hospital admission for myocardial infarction[156] or carotid endarterectomy.[157] Additionally, serum levels of anti-apoA-I could be used to predict the presence of coronary artery stenosis in obese but otherwise healthy individuals.[158]

Functionality of HDL as a potential biomarker in drug development and potential therapeutic target

Traditional HDL-directed therapies of cardiovascular diseases aimed at increasing HDL-cholesterol levels. However, as described above, HDL loses physiological function and gains pathological dysfunction in cardiovascular disease states. Therefore, it might be more effective

for the pharmacological treatment and prevention of cardiovascular diseases to improve the functionality of HDL particles rather than to increase the concentration of potentially dysfunctional HDL. As yet this hypothesis has been tested retrospectively on drugs that are already marketed (statins, fibrates, and niacin) as well as in accompanying research programs of therapies under development (CETP inhibitors and reconstituted HDL) (Table 3).

Statins

Statins reduce the burden of CHD mainly if not exclusively by lowering LDL-cholesterol.[1] They have little effect on HDL-cholesterol levels despite affecting the production rate and catabolism of HDL. There has been much interest in the effects of statin therapy on the functional properties of HDL in cardiovascular disease. As yet the information of clinical studies is controversial.

Treatment of healthy subjects with either pravastatin at a daily dose of 40 mg or atorvastatin at daily doses of 10 or 80 mg for 16 weeks was, on average, associated with a 14% increase in the functionality of HDL to protect LDL against copper-induced oxidation.[159] Moreover, 6-week therapy with 40 mg per day simvastatin in patients with coronary heart disease resulted in a significant improvement in the ability of HDL to inhibit oxidation and monocyte chemotactic activity released by cells after stimulation by oxidized LDL.[160] In a small study involving dyslipidemic patients, treatment with 2 mg pitavastatin for 4 weeks increased serum concentration of HDL-cholesterol levels by 9% and the HDL phospholipid content by 8%.[161] This was accompanied by similar increases in the capacity of the HDL fraction of serum to remove cholesterol from human macrophages and HDL-associated PON1 activity.[161] Equally, a 6-week long treatment with atorvastatin at 10 mg or 40 mg dose-dependently elevated cellular free cholesterol efflux towards plasma by 15% and 35%, respectively, in subjects with mixed dyslipidemia.[162] This improved cholesterol efflux capacity may be the result of the 24% increase in apoA-I levels observed following atorvastatin therapy.[162] Alternative reasons may

be the marked reductions in the CETP-mediated transfer of cholesteryl esters from HDL to apoB-containing lipoproteins,[162] which at least in animal models has been suggested to be atheroprotective.

In contrast to the aforementioned studies, 16 weeks of therapy with either 10 mg of atorvastatin, 80 mg of atorvastatin, or 40 mg of pravastatin did not improve the ability of HDL to promote cholesterol efflux from macrophages in CAD patients.[49] Niesor et al. even reported unfavorable properties of statins regarding HDL-facilitated cholesterol efflux.[163] Statin exposure of cholesterol-loaded human macrophages led to the upregulation of miR-33 mRNA expression, reducing both ABCA1 mRNA expression and ABCA1-mediated cholesterol efflux.[163] Interestingly, by the same mechanism, concomitant statin treatment compromised the positive effects of other HDL-raising drugs, such as the CETP-inhibitor dalcetrapib, on cholesterol efflux capacity.[163] Thus, one may speculate that the effects of statins on macrophage miR-33 expression interfere with the efficacy of CETP inhibitors in statin-treated patients.

Collectively, these observations account against large benefits of statin therapy on the functional properties of HDL, at least if mediated by cholesterol efflux.

Niacin

Niacin (also known as nicotinic acid or vitamin B3) is an effective agent to improve pro-atherogenic lipid profiles. In therapeutic doses, niacin can reduce LDL-cholesterol levels by 15-20% and plasma triglyceride levels by 25-40%, while raising HDL-cholesterol levels by 20-35%.[164] Moreover, extended-release niacin when added to statin monotherapy resulted in lowering of lipoprotein(a) and oxidized LDL as well as the inflammatory markers SAA and monocyte chemoattractant protein-1.[165] As a consequence, like statins, also niacin has attracted research interest as a therapeutic candidate for modifying HDL structure and function. In fact, mass spectrometry-based proteome analysis revealed that 1 year combination treatment with atorvastatin (10 to 20 mg daily) plus extended-release niacin (2 g daily) in CAD patients

remodeled the protein composition of HDL_3 particles toward that of healthy control subjects.[166] These alterations in HDL structure might contribute to some of the documented effects of combined niacin/statin therapy on HDL functions. Sorrentino et al. reported that administration of 1500 mg/day extended-release niacin for 3 months in type 2 diabetic patients restored the endothelial-vasoprotective properties of HDL and prevented the accumulation of both lipid oxidation products and MPO on HDL.[167] In correspondence with these findings, 6-week therapy with extended-release niacin (0.5 g/day) in individuals with the metabolic syndrome caused a moderate improvement in the ability of HDL to suppress VCAM-1 expression and increase eNOS protein abundance in endothelial cells.[168] Furthermore, apoB-depleted plasma from niacin-treated subjects was found to enhance macrophage cholesterol efflux capacity, although primarily explained by the elevated HDL-cholesterol concentrations.[169] However, several recent studies have postulated that niacin does not improve metrics of HDL functionality. No differences were observed in the protective effects of HDL against LDL oxidation between dyslipidemic patients treated with extended-release niacin/laropiprant on top of statin therapy and those treated with statin monotherapy.[165] An intervention study in patients with dyslipidemia found no significant change in serum cholesterol efflux capacity after 6 weeks of extended-release niacin therapy (titrated up to 1 g/day).[170] Similarly, adding niacin (2 g daily) to simvastatin therapy provided no additional benefit with regard to cholesterol efflux capacity and HDL inflammatory index in patients with carotid atherosclerosis, despite increasing HDL-cholesterol by 29%.[171]

A placebo-controlled clinical trial by Taylor et al. examined whether extended-release niacin slows the progression and/or causes regression of angiographically assessed coronary atherosclerosis.[172] In patients with CHD or high risk of CHD receiving long-term statin therapy, additional treatment with extended-release niacin at a target dose of 2 g daily resulted in a regression of mean carotid intima-media thickness over 14 months.[172] However, carotid regression of subclinical atherosclerosis induced by niacin therapy did not translate into better

clinical outcome in both the Atherothrombosis Intervention in Metabolic Syndrome with Low HDL/High Triglycerides: Impact on Global Health Outcomes (AIM-HIGH) trial[173] and the Heart Protection Study 2-Treatment of HDL to Reduce the Incidence of Vascular Events (HPS-THRIVE) trial.[174] A recent meta-analysis of 387 randomized-control trials implied that niacin is effective in reducing the risk of nonfatal myocardial infarction in individuals not treated with a statin, whereas on top of background statin treatment niacin failed to reduce cardiovascular events.[175] Thus, available evidence does not support the routine use of niacin and statin combination therapy for the prevention of cardiovascular disease events. The non-unequivocal outcomes of the HDL function tests may also reflect the lack of clinical efficacy.

Fibrates

As activators of the peroxisome proliferator activator receptor alpha (PPAR-α), fibrates regulate the expression of several genes with a pivotal role in HDL metabolism, notably apoA-I, ABCA1 (indirectly via the liver X receptor), phospholipid transfer protein, and SR-BI. Probably because the induction of these genes that exert opposite effects on HDL-cholesterol levels, fibrate treatment only moderately increases HDL-cholesterol levels. The HDL-cholesterol increasing effect is enhanced in patients with elevated triglycerides, which are lowered by fibrate treatment. The addition of fenofibrate to statins decreased cardiovascular event rates neither in the FIELD trial nor in the ACCORD study, which both included patients with diabetes and dyslipidemia.[176, 177] According to a recent meta-analysis, fibrates like niacin are effective in reducing the risk of nonfatal myocardial infarction in individuals not treated with a statin.[175] However, unlike for niacin post hoc analyses identified a subgroup of patients with both low HDL-cholesterol and hypertriglyceridemia who experienced one third less events when treated with a combination of statins with fibrates rather than with fibrates alone.[178] Also in the absence of statins, this subgroup appears to benefit over-proportionately from treatment with bezafibrate or gemfibrozil.[178]

Treatment of patients with fenofibrate, bezafibrate, ciprofibrate, or the potent and selective PPAR-α agonist LY518674 increased the capacity of total or apoB-free plasma to release cholesterol from macrophages in some studies,[179-183] but not in others.[170, 184] Potential reasons are differences in patient populations and cholesterol efflux assays. Notably, ABCA1-mediated cholesterol efflux capacity appears to be improved upon fibrate treatment. Interestingly, under treatment with LY518674 the changes in ABCA1-mediated cholesterol efflux capacity and apoA-I production were found to correlate with one another.[179] In mice transgenic for human apoA-I, treatment with gemfibrozil or fenofibrate increased the reverse transport from macrophages to feces.[185]

The effect of fibrate treatment on the anti-oxidative, anti-inflammatory, or endothelial functionality of HDL has been less extensively investigated. In diabetic subjects, ciprofibrate treatment did not significantly change the ability of HDL to inhibit the oxidation of LDL or the expression of monocyte chemotactic protein-1 by human umbilical vein endothelial cells.[180] In a cross-over-study of 33 patients with mixed hyperlipidemia treatments with fenofibrate and niacin were both found to improve the capacity of HDL to enhance nitric oxide production and inhibit VCAM-1 expression by endothelial cells.[168] Several investigators found that fenofibrate treatment increases the activity and concentration of PON1.[184, 186, 187]

CETP inhibitors

CETP inhibitors including torcetrapib, dalcetrapib, evacetrapib, and anacetrapib increase HDL-cholesterol levels by up to 60%, 30%, 120%, and 140%, respectively, and lower LDL-cholesterol by up to 20%, 0%, 30%, and 40%, respectively. Despite their beneficial effects on these lipoprotein traits, three of four large randomized controlled outcome trials on the clinical efficacy of add-on-statin therapy with CETP inhibitors have been stopped prematurely because of excess cardiovascular and overall mortality (torcetrapib) or futility (dalcetrapib and evacetrapib).[188, 189, 190] Anacetrapib is the only CETP inhibitor, which is under ongoing phase

3 trial investigation for efficacy in preventing clinical endpoints. In the DEFINE (Determining the Efficacy and Tolerability of CETP Inhibition with AnacEtrapib) trial including 1623 patients with known cardiovascular heart disease or at high risk of CHD, co-administration of anacetrapib 100 mg once daily with a statin for 24 weeks provided an additional 40% reduction in LDL-cholesterol and 138% increase in HDL-cholesterol compared with the statin plus placebo group,[191] irrespective of the patient subgroup.[192] A post hoc analysis found that 2.0% of anacetrapib- and 2.6% of placebo-treated subjects experienced a primary end-point at 76 weeks.[191] The ongoing Randomized Evaluation of the Effects of Anacetrapib Through Lipid-modification (REVEAL) trial that aims to assess whether anacetrapib (100 mg/day) reduces cardiovascular events among statin-treated patients with a history of cardiovascular disease is ongoing. The results of this study will have to awaited before one can conclude whether CETP inhibition in general is the wrong strategy or whether the specific compound or patient groups determines clinical utility of CETP inhibition.

The adverse effect of torcetrapib has been explained by off-target effects on blood pressure. This was not seen for dalcetrapib or evacetrapib so that their futility to prevent cardiovascular disease events is still not understood. Investigations of both monogenic CETP deficiency and polymorphisms in the CETP gene provided controversial evidence. Some studies reported reduced cardiovascular disease risk and prolonged life expectancy in individuals with CETP mutants,[193-196] others did not find any impact or even increased cardiovascular risk.[197, 198] Previous meta-analyses of genetic studies, provided compelling evidence that polymorphisms that are associated with reduced CETP activity and increased HDL-cholesterol levels are also associated with reduced cardiovascular risk.[199, 200] Another recent meta-analysis of this Mendelian randomization approach, however, conversely found, that a specific increase in circulating HDL-cholesterol levels due to the Taq1B polymorphism in the CETP gene was not associated with a lower risk of developing CAD.[201] Population studies that investigated CETP plasma concentrations as a biomarker also yielded results that contradict the initial genetic

studies: Low rather than high CETP concentrations were associated with increased cardiovascular mortality in a nearly 8 years long follow-up of more than 3000 patients who underwent coronary angiography.[202] In the PROVE IT-TIMI 22 study, plasma CETP levels were not associated with differences in cardiovascular event rates. In the 50% of patients who reached the lowest concentrations of LDL-cholesterol by pravastatin treatment, a low CETP concentration was associated with excess cardiovascular event rates.[203] It hence remains questionable whether lowering of CETP activity at least later in life or in combination with statin therapy reduces cardiovascular risk despite increasing HDL-cholesterol and decreasing LDL-cholesterol.

Animal models provided controversial data on the role of CETP in atherosclerosis. Wild-type mice lack CETP, so that only the effect of transgenic CETP overexpression on atherosclerosis could be investigated. In wild-type mice as well as in hypercholesterolemic mouse models, overexpression of CETP increased atherosclerosis.[204, 205] By contrast, in hypertriglyceridemic mouse models and in mice overexpressing LCAT, CETP overexpression reduced atherosclerosis despite lowering HDL-cholesterol.[204, 205] In rabbits, vaccination against CETP[206] as well as CETP inhibition with either JTT705 (= dalcetrapib),[207] torcetrapib,[208] or antisense-nucleotides[209] were found to increase HDL-cholesterol and reduce atherosclerosis.

Most studies regarding HDL functionality investigated the impact of CETP inhibitors on cholesterol efflux capacity as well as the delivery of cholesteryl esters to hepatocytes *in vitro* as well as macrophage reverse cholesterol transport in animals. In general these activities were improved by the application of a CETP inhibitor. There is limited information on the impact of CETP inhibition on anti-inflammatory or endothelial functionalities of HDL.

Torcetrapib: Cholesterol efflux from THP-1 macrophages to apoB-depleted plasma of subjects with moderate hypercholesterolemia without cardiovascular disease was higher among patients treated with 60 or 120 mg torcetrapib for 8 weeks, and at least for the higher dose this effect was independent of increases in HDL-cholesterol levels.[210] In agreement with these

observations, in male subjects with high-risk HDL-cholesterol levels (<40 mg/dl), 6-week combination treatment with torcetrapib (60 mg/day) and atorvastatin (10 mg/day) enhanced the capacity of large HDL_2 particles to mediate cholesterol efflux via SR-BI and the delivery of HDL cholesteryl esters to hepatoma cells when compared to atorvastatin monotherapy.[211] Similar findings were reported from a study in patients with mixed hyperlipidemia where HDL_2-mediated cholesterol efflux and uptake of HDL cholesteryl esters by hepatocytes were shown to be augmented by a 6-week period of combined torcetrapib/atorvastatin (60/10 mg/day) therapy.[212] The treatment of hamsters with torcetrapib increased the mobilization of radiolabeled cholesterol from peritoneal macrophages into plasma HDL and subsequent excretion into stool.[213, 214]

Dalcetrapib: Results of a phase IIb study comparing different doses of dalcetrapib in dyslipidemic patients treated with pravastatin (40 mg/day) indicated a positive effect of dalcetrapib on ABCA1- and SR-BI-mediated cellular cholesterol removal.[215] Likewise, in the dal-ACUTE study that enrolled 300 patients who had a recent ACS, dalcetrapib at a daily dose of 600 mg enhanced the capacity of apoB-depleted plasma to elicit non-ABCA1-specific cholesterol efflux from macrophage foam cells, which was related to changes in apoA-I and HDL-cholesterol levels.[216] In normolipidemic hamsters, dalcetrapib promoted the fecal elimination of macrophage-derived cholesterol,[214] but in high-fat-induced hyperlipidemic hamsters the effect was in the opposite direction, with dalcetrapib limiting the excretion of macrophage cholesterol.[217] Dalcetrapib treatment did not restore the reduced ability of HDL from CHD patients to stimulate endothelial nitric oxide production and to inhibit apoptosis and VCAM-1 expression of endothelial cells.[218] In a recent post hoc analysis of the dal-OUTCOMES trial, Tardif et al. revealed that genetic differences in the adenylate cyclase 9 (ADCY9*)* locus stratifies recipients of dalcetrapib towards cardiovascular outcome.[219] Dalcetrapib treatment decreased the cardiovascular event rate by 39% in patients with the AA genotype at rs1967309 but increased cardiovascular event rates by 27% in patients with the GG

genotype.[219] The ADCY9 gene encodes an adenylate cyclase that catalyzes the formation of the signaling molecule cAMP from ATP. Since cAMP regulates ABCA1, Tardiff et al. hypothesized that the ADCY9 gene *trans*-regulates ABCA1 activity, which may in turn determine the translation of dalcetrapib-induced changes in HDL metabolism into cardiovascular efficacy. Alternatively, since adenylate cyclases are downstream targets of G-protein-coupled receptors (GPCRs) and since HDL components such as S1P interact with GPCRs, it is tempting to speculate that HDL-induced signaling pathways could be potentially affected by the polymorphism in the ADCY9 gene. Depending on the functionality of the agonist, the mutation may promote beneficial effects of physiological HDL or abrogate adverse effects of dysfunctional HDL.

Anacetrapib: HDL from dyslipidemic patients, who received anacetrapib 300 mg/day for 8 weeks, were more potent at promoting net transfer of cholesterol out of macrophage foam cells than HDL from placebo-treated patients.[169] These results are consistent with the stimulatory effects of anacetrapib on macrophage-to-feces reverse cholesterol transport in both normolipidemic and dyslipidemic hamsters.[217, 220] Anacetrapib treatment in dyslipidemic hamsters and healthy human volunteers had no effect on the ability of HDL to blunt cytokine-induced expression of adhesion molecules and monocyte chemotactic protein-1 in endothelial cells.[221]

Overall these data indicate that CETP inhibition improves cholesterol efflux capacity of plasma but does not alter anti-inflammatory or endothelium-protective activities of HDL.

HDL mimetics

HDL-like discoidal particles can be artificially reconstituted by the use of water soluble apolipoproteins, notably apoA-I or apoE, or even amphipathic peptides.[7, 222, 223] In cell culture experiments these rHDL particles imitate several potentially anti-atherogenic effects of native HDL, notably the induction of cholesterol efflux from macrophage foam cells as well as the

stimulation of nitric oxide production and the inhibition of VCAM-1 expression and apoptosis by endothelial cells. In animal models of atherosclerosis, infusion of rHDL prevented the development of atherosclerosis or even caused regression of atherosclerosis.[222, 223] At least three formulations of rHDL have entered clinical development and have been applied to humans:

- CSL111 and its successor CSL112 consist of native apoA-I and phosphatidylcholines isolated from human plasma and soybean, respectively.[7, 222, 223] In the ERASE trial of 186 patients with ACS 2 weeks before, four weekly infusions of 40 mg CSL111/kg body weight induced a reduction of coronary atheroma volume as assessed by intravascular ultrasound (IVUS), which was statistical significant compared to baseline but not compared to placebo.[224] The infusion of CSL111 was also found to improve endothelial dysfunction, platelet function, and glycaemia in patients with type 2 diabetes mellitus.[32, 225-227] Both total and apoB-free plasma of probands infused with CSL111 showed increased cholesterol efflux capacity.[228, 229] The relative increase in cholesterol efflux capacity was even higher in total plasma than in apoB-free plasma (the surrogate of HDL),[230] reinforcing the previous notion that apoB-containing lipoproteins enhance the cholesterol efflux elicited by HDL through cycling of cell-derived cholesterol between cells and HDL.[229, 230] As lipid-containing particles, rHDL directly enhances cholesterol efflux via the SR-BI and ABCG1-mediated pathways. Indirectly however, it also appears to enhance ABCA1-mediated cholesterol efflux. Phase 1 studies on the second generation product CSL112 revealed that the infusion of rHDL increases the formation of prebeta-HDL (that is lipid-free apoA-I) by a factor 36 and, probably as the result, the capacity of plasma to elicit ABCA1-mediated cholesterol efflux *in vitro* by 270%.[231] HDL from plasma of probands treated with CSL111 showed increased anti-inflammatory capacities to inhibit the expression of VCAM-1 and ICAM-1 by endothelial cells as well as the expression of CD11b by monocytes and the adhesion of neutrophils to a fibrinogen matrix.[228]

- ETC-215 which is now further developed as MDCO-216 contains the apoA-I(R173C)$_{Milano}$ variant and palmitoyloleylcholine.[7, 222, 223] Compared to wild-type apoA-I containing rHDL, rHDL with apoA-I(R173C)$_{Milano}$ showed more potent cholesterol efflux-stimulating as well as anti-inflammatory capacities.[232] Like rHDL containing wild type apoA-I, ETC-215 induced the regression of atherosclerosis in both rabbit and mouse models of atherosclerosis.[7, 222, 223] In a phase 2 study of 57 patients, five weekly ETC-215 infusions two weeks after ACS caused significant regression of IVUS-assessed total atheroma volume in coronary arteries compared to baseline.[233] Infusion of MDCO-216 into cynomolgous monkeys increased the capacity of plasma to induce cholesterol efflux via the ABCA1 pathway much more prominently than via the ABCG1 and SR-BI pathways, probably by displacing endogenous wild-type apoA-I from endogenous HDL and thereby enhancing the formation of prebeta1-HDL.[234] A similar effect was seen *ex vivo*, when MDCO-216 was mixed with human plasma.[235]
- CER-001 is the third formulation of rHDL that has entered clinical evaluation in humans. It contains recombinant apoA-I, diphosphatidylglycerol, and sphingomyelin.[236] The presence of sphingomyelin appears to enhance the cholesterol efflux capacity of both the rHDL itself and the plasma containing rHDL.[236] In an open label study of seven patients with familial low HDL cholesterol syndromes (familial hypoalphalipoproteinemia), 9 infusions of 8 mg/kg body weight CER-001 led to a significant regression of carotid atherosclerosis as assessed by NMR.[237] At the same time the cholesterol efflux capacity of plasma was significantly increased compared to baseline. Also the fecal sterol excretion was increased, however not significantly (P = 0.068).[237] In 23 patients with homozygous familial hypercholesterolemia, 12 biweekly infusions of CER-001 led to a significant reduction in carotid mean vessel wall area after 24 weeks.[238] In a randomized controlled trial of 500 ACS patients six weekly infusions of placebo, 3 mg/kg, 6 mg/kg, or 12 mg/kg CER-001 had significant impact

neither on IVUS assessed coronary atheroma volume nor angiography assessed severity or extent of CHD.[239]

Conclusions

HDL functionality has potential implications for diagnostics as well as therapy and hence biomarker discovery and drug development.

Diagnostics and biomarker discovery

Because of the failures of the recent trials on fenofibrate, niacin, torcetrapib, dalcetrapib, and most recently evacetrapib, but also because of lacking genetic association of HDL cholesterol with cardiovascular risk, changes in HDL cholesterol are no longer considered as a surrogate marker for drug development. Previously, HDL function has rather been a subordinate endpoint in drug development. This appears to change, although there is as yet no evidence on the ideal HDL function test. The exact role and hence clinical importance of dysfunctional HDL in the pathogenesis of cardiovascular diseases is still unclear because of three main reasons:

First, most dysfunctions have been identified by small exploratory case-control studies which aimed at the identification of causes and effects of HDL dysfunction on the molecular level. With the exception of cholesterol efflux capacity no dysfunction has been investigated in large cross-sectional or even longitudinal studies for diagnostic and prognostic performance, respectively. To this end the HDL function tests must be not only technically feasible for high throughput, but also analytically robust, and pre-analytically controlled. As yet only data on the analytical performance of cholesterol efflux capacity, and HDL-induced nitric oxide and superoxide production in endothelial cells as well as serum PON1 activity have been published. These assays appear to work reproducibly at least within individual specialized labs.[49-51, 240] Accuracy is difficult to assess because there is no gold standard. Cholesterol efflux capacity appears to be strongly influenced by the method used.[50, 51] For other functions no method

comparisons have been reported. The intraindividual variability of cholesterol efflux capacity, HDL-induced nitric oxide and superoxide production in endothelial cells as well as serum PON1 activity within one day, one week and one month was found very low.[49, 51, 240] Neither did sample storage affect the measurement of cholesterol efflux capacity significantly.[49] Graded by stages of biomarker development[241] and in comparison with established and other emerging biomarkers of HDL metabolism, HDL function tests are in a rather early phase of development with cholesterol efflux capacity being most advanced (Table 4).

Second, the relative importance of the many functions of normal HDL from healthy individuals and many dysfunctions of pathological HDL for the pathogenesis of atherosclerosis is not known. The present scientific literature is dominated by studies that record cholesterol efflux capacity. This does not only reflect the fact that the mediation of cholesterol efflux is the classical anti-atherogenic function but also feasibility: it is performed on total or apoB-free serum and thereby avoids the laborious and also artefact-prone isolation of HDL by ultracentrifugation.[242] However, the increase in cholesterol efflux capacity but lack of clinical efficacy by treatment with torcetrapib and dalcetrapib (cf. Table 3) indirectly questions the feasibility of this bioassay to aid in drug development or monitor treatment success. The unchanged endothelial and anti-inflammatory functionalities of HDL appear to be in better accordance. Systematic head to head comparisons are needed to establish the HDL functionality which has the strongest association with CHD and treatment response and is therefore likely most relevant for the anti-atherogenicity of HDL.

Third, structure-function-relationships of HDL function and dysfunction are not comprehensively elaborated. There are many examples of functions and dysfunctions that have been associated with different molecules within HDL. For example the ability of normal HDL to stimulate nitric oxide production in endothelial cells has been assigned to pure apoA-I/phospholipid complexes [222, 223] as well as to minor constituents such as S1P.[20] Endothelial HDL dysfunction has been assigned to the loss of S1P,[119] oxidative modifications of apoA-I or

phospholipids,[40] enrichment with SAA[65] or SDMA.[41] Likewise, differences in cholesterol efflux capacity have been attributed to differences in HDL particle number, HDL subclass distribution, phospholipid composition,[86] the presence of SAA,[58-61] or posttranslational modifications.[127-132] As yet it is not clear whether these findings reflect redundancy or complexity of HDL interactions with endothelial cells or result from confounding.

The solution of these three general problems is pivotal for the targeting of HDL dysfunction by both biomarkers for improved diagnostics and therapy. Systems biological approaches with comprehensive characterization of HDL structure and composition as well as functionality are needed to resolve this question. Such an approach may lead to the identification of pivotal molecules that to a large extent differentiate functional and dysfunctional HDL and can hence be used as biomarkers for drug development and monitoring of treatment success.

Therapy and drug development

The active components and the downstream cellular responders of both functional HDL and dysfunctional HDL are interesting targets for drug development towards treatment or prevention of CHD. In this regard it is again pivotal to establish the most relevant functions and the structural correlates of HDL in the pathogenesis of atherosclerosis. Mediators of physiological protective functions would need activation, whereas mediators of HDL dysfunction would need inhibition. It is hence crucial to establish whether changes in the functionality of HDL under inflammatory conditions are caused by loss of function or gain of dysfunction (Figure 2). Loss of functional HDL components would need replenishment or activation. Gain of dysfunction will require the elimination of harmful components or blockage of their formation and their cellular responders. In this situation, although at first sight paradoxically, therapies that enhance the catabolism of HDL and thereby lower HDL cholesterol, such as probucol[243] or testosterone,[244] will be rather beneficial. Such therapies may benefit from combination with HDL mimetics or stimulators of HDL production. Also drugs

that induce both HDL production and HDL catabolism, such as fibrates or statins, will be good candidates although they do not alter HDL-cholesterol levels much. Any therapy that prolongs the residence time of HDL with gained adverse function, for example CETP inhibitors or estrogens, will be damaging rather than beneficial, although they increase HDL-cholesterol levels.

Legends to the figures

Figure 1: Principle physiological structure-function-relationships of normal HDL. HDL are heterogenous particles differing by size shape and composition of proteins, lipids, and microRNAs. HDL and its components elicit cellular responses either by modulating cholesterol homeostasis through cholesterol efflux or by specific interactions with signalling receptors (for example activation of S1P receptors by S1P or the ecto-ATPase/purinergic receptor axis by apoA-I). Either principle mechanism can lead to posttranslational short term effects or rather transcriptional long term effects and thereby alter the function and survival of cells. ABC's = ATP-binding cassette transporters; GPCR = G-protein coupled receptor; SR-BI = scavenger receptor class B type I.

Figure 2: Principle structure-function-relationships of pathological HDL. Modifications in the composition of HDL or structural alterations of the HDL components can produce either loss of physiological functions or gain of pathological dysfunction. The former compromises the physiological interactions with cells described in Figure 1. The latter generates novel interactions, for example with pattern recognition receptors such as toll-like receptor 2 (TLR2) or lectin-like oxidized receptor 1 (LOX-1). For comparison and explanations of other abbreviations see Figure 1.

Acknowledgements

Conflicts of Interest: All authors have read the journal's policy on disclosure of potential conflicts of interest and have none to declare.

Wijtske Annema was supported by funding from the Ter Meulen Fund, Royal Netherlands Academy of Arts and Sciences. Arnold von Eckardstein's research on HDL is currently supported by grants from the Swiss National Science Foundation (31003A-160126/1 and CRSII3_154420/1), the Swiss Systems X program (HDL-X), and the 7th Framework Program of the European Commission ("RESOLVE", Project number 305707 and "TransCard", Project number 603091).

All authors have read the journal's authorship agreement and approved the manuscript.

References

1. Cholesterol Treatment Trialists C, Fulcher J, O'Connell R, et al. Efficacy and safety of LDL-lowering therapy among men and women: meta-analysis of individual data from 174,000 participants in 27 randomised trials. Lancet. 2015;385:1397-1405.
2. Emerging Risk Factors C, Di Angelantonio E, Sarwar N, et al. Major lipids, apolipoproteins, and risk of vascular disease. JAMA. 2009;302:1993-2000.
3. Hoekstra M, Van Eck M. Mouse models of disturbed HDL metabolism. Handb Exp Pharmacol. 2015;224:301-336.
4. Luscher TF, Landmesser U, von Eckardstein A, Fogelman AM. High-density lipoprotein: vascular protective effects, dysfunction, and potential as therapeutic target. Circ Res. 2014;114:171-182.
5. Annema W, von Eckardstein A. High-density lipoproteins. Multifunctional but vulnerable protections from atherosclerosis. Circ J. 2013;77:2432-2448.
6. Voight BF, Peloso GM, Orho-Melander M, et al. Plasma HDL cholesterol and risk of myocardial infarction: a mendelian randomisation study. Lancet. 2012;380:572-580.
7. Darabi M, Guillas-Baudouin I, Le Goff W, Chapman MJ, Kontush A. Therapeutic applications of reconstituted HDL: When structure meets function. Pharmacol Ther. 2015
8. Kontush A, Lindahl M, Lhomme M, et al. Structure of HDL: particle subclasses and molecular components. Handb Exp Pharmacol. 2015;224:3-51.
9. Vickers KC, Palmisano BT, Shoucri BM, Shamburek RD, Remaley AT. MicroRNAs are transported in plasma and delivered to recipient cells by high-density lipoproteins. Nat Cell Biol. 2011;13:423-433.

10. Wagner J, Riwanto M, Besler C, et al. Characterization of levels and cellular transfer of circulating lipoprotein-bound microRNAs. Arterioscler Thromb Vasc Biol. 2013;33:1392-1400.

11. Riwanto M, Landmesser U. High density lipoproteins and endothelial functions: mechanistic insights and alterations in cardiovascular disease. J Lipid Res. 2013;54:3227-3243.

12. Besler C, Luscher TF, Landmesser U. Molecular mechanisms of vascular effects of High-density lipoprotein: alterations in cardiovascular disease. EMBO Mol Med. 2012;4:251-268.

13. Annema W, von Eckardstein A, Kovanen PT. HDL and atherothrombotic vascular disease. Handb Exp Pharmacol. 2015;224:369-403.

14. Cuchel M, Rader DJ. Macrophage reverse cholesterol transport: key to the regression of atherosclerosis? Circulation. 2006;113:2548-2555.

15. Annema W, Tietge UJ. Regulation of reverse cholesterol transport - a comprehensive appraisal of available animal studies. Nutr Metab (Lond). 2012;9:25.

16. Navab M, Hama SY, Anantharamaiah GM, et al. Normal high density lipoprotein inhibits three steps in the formation of mildly oxidized low density lipoprotein: steps 2 and 3. J Lipid Res. 2000;41:1495-1508.

17. Navab M, Hama SY, Cooke CJ, et al. Normal high density lipoprotein inhibits three steps in the formation of mildly oxidized low density lipoprotein: step 1. J Lipid Res. 2000;41:1481-1494.

18. Kontush A, Chantepie S, Chapman MJ. Small, dense HDL particles exert potent protection of atherogenic LDL against oxidative stress. Arterioscler Thromb Vasc Biol. 2003;23:1881-1888.

19. Watson AD, Berliner JA, Hama SY, et al. Protective effect of high density lipoprotein associated paraoxonase. Inhibition of the biological activity of minimally oxidized low density lipoprotein. J Clin Invest. 1995;96:2882-2891.

20. Nofer JR, van der Giet M, Tolle M, et al. HDL induces NO-dependent vasorelaxation via the lysophospholipid receptor S1P3. J Clin Invest. 2004;113:569-581.

21. Riwanto M, Rohrer L, Roschitzki B, et al. Altered activation of endothelial anti- and proapoptotic pathways by high-density lipoprotein from patients with coronary artery disease: role of high-density lipoprotein-proteome remodeling. Circulation. 2013;127:891-904.

22. de Souza JA, Vindis C, Negre-Salvayre A, et al. Small, dense HDL 3 particles attenuate apoptosis in endothelial cells: pivotal role of apolipoprotein A-I. J Cell Mol Med. 2010;14:608-620.

23. Argraves KM, Gazzolo PJ, Groh EM, et al. High density lipoprotein-associated sphingosine 1-phosphate promotes endothelial barrier function. J Biol Chem. 2008;283:25074-25081.

24. Wilkerson BA, Grass GD, Wing SB, Argraves WS, Argraves KM. Sphingosine 1-phosphate (S1P) carrier-dependent regulation of endothelial barrier: high density lipoprotein (HDL)-S1P prolongs endothelial barrier enhancement as compared with albumin-S1P via effects on levels, trafficking, and signaling of S1P1. J Biol Chem. 2012;287:44645-44653.

25. Seetharam D, Mineo C, Gormley AK, et al. High-density lipoprotein promotes endothelial cell migration and reendothelialization via scavenger receptor-B type I. Circ Res. 2006;98:63-72.

26. Zhu W, Saddar S, Seetharam D, et al. The scavenger receptor class B type I adaptor protein PDZK1 maintains endothelial monolayer integrity. Circ Res. 2008;102:480-487.

27. Cockerill GW, Rye KA, Gamble JR, Vadas MA, Barter PJ. High-density lipoproteins inhibit cytokine-induced expression of endothelial cell adhesion molecules. Arterioscler Thromb Vasc Biol. 1995;15:1987-1994.

28. Nicholls SJ, Dusting GJ, Cutri B, et al. Reconstituted high-density lipoproteins inhibit the acute pro-oxidant and proinflammatory vascular changes induced by a periarterial collar in normocholesterolemic rabbits. Circulation. 2005;111:1543-1550.

29. Murphy AJ, Woollard KJ, Hoang A, et al. High-density lipoprotein reduces the human monocyte inflammatory response. Arterioscler Thromb Vasc Biol. 2008;28:2071-2077.

30. Bursill CA, Castro ML, Beattie DT, et al. High-density lipoproteins suppress chemokines and chemokine receptors in vitro and in vivo. Arterioscler Thromb Vasc Biol. 2010;30:1773-1778.

31. Tolle M, Pawlak A, Schuchardt M, et al. HDL-associated lysosphingolipids inhibit NAD(P)H oxidase-dependent monocyte chemoattractant protein-1 production. Arterioscler Thromb Vasc Biol. 2008;28:1542-1548.

32. Calkin AC, Drew BG, Ono A, et al. Reconstituted high-density lipoprotein attenuates platelet function in individuals with type 2 diabetes mellitus by promoting cholesterol efflux. Circulation. 2009;120:2095-2104.

33. Nofer JR, Walter M, Kehrel B, et al. HDL3-mediated inhibition of thrombin-induced platelet aggregation and fibrinogen binding occurs via decreased production of phosphoinositide-derived second messengers 1,2-diacylglycerol and inositol 1,4,5-tris-phosphate. Arterioscler Thromb Vasc Biol. 1998;18:861-869.

34. Brodde MF, Korporaal SJ, Herminghaus G, et al. Native high-density lipoproteins inhibit platelet activation via scavenger receptor BI: role of negatively charged phospholipids. Atherosclerosis. 2011;215:374-382.

35. Griffin JH, Kojima K, Banka CL, Curtiss LK, Fernandez JA. High-density lipoprotein enhancement of anticoagulant activities of plasma protein S and activated protein C. J Clin Invest. 1999;103:219-227.

36. Sugatani J, Miwa M, Komiyama Y, Ito S. High-density lipoprotein inhibits the synthesis of platelet-activating factor in human vascular endothelial cells. J Lipid Mediat Cell Signal. 1996;13:73-88.

37. Viswambharan H, Ming XF, Zhu S, et al. Reconstituted high-density lipoprotein inhibits thrombin-induced endothelial tissue factor expression through inhibition of RhoA and stimulation of phosphatidylinositol 3-kinase but not Akt/endothelial nitric oxide synthase. Circ Res. 2004;94:918-925.

38. Oslakovic C, Krisinger MJ, Andersson A, et al. Anionic phospholipids lose their procoagulant properties when incorporated into high density lipoproteins. J Biol Chem. 2009;284:5896-5904.

39. Riwanto M, Rohrer L, von Eckardstein A, Landmesser U. Dysfunctional HDL: from structure-function-relationships to biomarkers. Handb Exp Pharmacol. 2015;224:337-366.

40. Besler C, Heinrich K, Rohrer L, et al. Mechanisms underlying adverse effects of HDL on eNOS-activating pathways in patients with coronary artery disease. J Clin Invest. 2011;121:2693-2708.

41. Speer T, Rohrer L, Blyszczuk P, et al. Abnormal high-density lipoprotein induces endothelial dysfunction via activation of Toll-like receptor-2. Immunity. 2013;38:754-768.

42. Kim JB, Hama S, Hough G, et al. Heart failure is associated with impaired anti-inflammatory and antioxidant properties of high-density lipoproteins. Am J Cardiol. 2013;112:1770-1777.

43. Patel PJ, Khera AV, Wilensky RL, Rader DJ. Anti-oxidative and cholesterol efflux capacities of high-density lipoprotein are reduced in ischaemic cardiomyopathy. Eur J Heart Fail. 2013;15:1215-1219.

44. Singh N, Jacobs F, Rader DJ, et al. Impaired cholesterol efflux capacity and vasculoprotective function of high-density lipoprotein in heart transplant recipients. J Heart Lung Transplant. 2014;33:499-506.

45. Sviridov D, Chin-Dusting J, Nestel P, et al. Elevated HDL cholesterol is functionally ineffective in cardiac transplant recipients: evidence for impaired reverse cholesterol transport. Transplantation. 2006;81:361-366.

46. Bellanger N, Orsoni A, Julia Z, et al. Atheroprotective reverse cholesterol transport pathway is defective in familial hypercholesterolemia. Arterioscler Thromb Vasc Biol. 2011;31:1675-1681.

47. Ottestad IO, Halvorsen B, Balstad TR, et al. Triglyceride-rich HDL3 from patients with familial hypercholesterolemia are less able to inhibit cytokine release or to promote cholesterol efflux. J Nutr. 2006;136:877-881.

48. Balstad TR, Holven KB, Ottestad IO, et al. Altered composition of HDL3 in FH subjects causing a HDL subfraction with less atheroprotective function. Clin Chim Acta. 2005;359:171-178.

49. Khera AV, Cuchel M, de la Llera-Moya M, et al. Cholesterol efflux capacity, high-density lipoprotein function, and atherosclerosis. N Engl J Med. 2011;364:127-135.

50. Li XM, Tang WH, Mosior MK, et al. Paradoxical association of enhanced cholesterol efflux with increased incident cardiovascular risks. Arterioscler Thromb Vasc Biol. 2013;33:1696-1705.

51. Rohatgi A, Khera A, Berry JD, et al. HDL cholesterol efflux capacity and incident cardiovascular events. N Engl J Med. 2014;371:2383-2393.

52. Ritsch A, Scharnagl H, Marz W. HDL cholesterol efflux capacity and cardiovascular events. N Engl J Med. 2015;372:1870-1871.

53. Saleheen D, Scott R, Javad S, et al. Association of HDL cholesterol efflux capacity with incident coronary heart disease events: a prospective case-control study. Lancet Diabetes Endocrinol. 2015;3:507-513.

54. Annema W, Dikkers A, de Boer JF, et al. HDL Cholesterol Efflux Predicts Graft Failure in Renal Transplant Recipients. J Am Soc Nephrol. 2015

55. Alwaili K, Bailey D, Awan Z, et al. The HDL proteome in acute coronary syndromes shifts to an inflammatory profile. Biochim Biophys Acta. 2012;1821:405-415.

56. Clifton PM, Mackinnon AM, Barter PJ. Effects of serum amyloid A protein (SAA) on composition, size, and density of high density lipoproteins in subjects with myocardial infarction. J Lipid Res. 1985;26:1389-1398.

57. Coetzee GA, Strachan AF, van der Westhuyzen DR, et al. Serum amyloid A-containing human high density lipoprotein 3. Density, size, and apolipoprotein composition. J Biol Chem. 1986;261:9644-9651.

58. Banka CL, Yuan T, de Beer MC, et al. Serum amyloid A (SAA): influence on HDL-mediated cellular cholesterol efflux. J Lipid Res. 1995;36:1058-1065.

59. van der Westhuyzen DR, Cai L, de Beer MC, de Beer FC. Serum amyloid A promotes cholesterol efflux mediated by scavenger receptor B-I. J Biol Chem. 2005;280:35890-35895.

60. Artl A, Marsche G, Lestavel S, Sattler W, Malle E. Role of serum amyloid A during metabolism of acute-phase HDL by macrophages. Arterioscler Thromb Vasc Biol. 2000;20:763-772.

61. Vaisar T, Tang C, Babenko I, et al. Inflammatory remodeling of the HDL proteome impairs cholesterol efflux capacity. J Lipid Res. 2015;56:1519-1530.

62. Annema W, Nijstad N, Tolle M, et al. Myeloperoxidase and serum amyloid A contribute to impaired in vivo reverse cholesterol transport during the acute phase response but not group IIA secretory phospholipase A(2). J Lipid Res. 2010;51:743-754.

63. Cai L, de Beer MC, de Beer FC, van der Westhuyzen DR. Serum amyloid A is a ligand for scavenger receptor class B type I and inhibits high density lipoprotein binding and selective lipid uptake. J Biol Chem. 2005;280:2954-2961.

64. Tolle M, Huang T, Schuchardt M, et al. High-density lipoprotein loses its anti-inflammatory capacity by accumulation of pro-inflammatory-serum amyloid A. Cardiovasc Res. 2012;94:154-162.

65. Zewinger S, Drechsler C, Kleber ME, et al. Serum amyloid A: high-density lipoproteins interaction and cardiovascular risk. Eur Heart J. 2015

66. Ashby D, Gamble J, Vadas M, et al. Lack of effect of serum amyloid A (SAA) on the ability of high-density lipoproteins to inhibit endothelial cell adhesion molecule expression. Atherosclerosis. 2001;154:113-121.

67. Van Lenten BJ, Hama SY, de Beer FC, et al. Anti-inflammatory HDL becomes pro-inflammatory during the acute phase response. Loss of protective effect of HDL against LDL oxidation in aortic wall cell cocultures. J Clin Invest. 1995;96:2758-2767.

68. Chiba T, Chang MY, Wang S, et al. Serum amyloid A facilitates the binding of high-density lipoprotein from mice injected with lipopolysaccharide to vascular proteoglycans. Arterioscler Thromb Vasc Biol. 2011;31:1326-1332.

69. Vaisar T, Mayer P, Nilsson E, et al. HDL in humans with cardiovascular disease exhibits a proteomic signature. Clin Chim Acta. 2010;411:972-979.

70. Xiong X, Liu H, Hua L, et al. The association of HDL-apoCIII with coronary heart disease and the effect of statin treatment on it. Lipids Health Dis. 2015;14:127.

71. Chang PY, Lee CM, Hsu HC, et al. Identification of the HDL-ApoCIII to VLDL-ApoCIII ratio as a predictor of coronary artery disease in the general population: the

Chin-Shan Community Cardiovascular Cohort (CCCC) study in Taiwan. Lipids Health Dis. 2012;11:162.

72. Kavo AE, Rallidis LS, Sakellaropoulos GC, et al. Qualitative characteristics of HDL in young patients of an acute myocardial infarction. Atherosclerosis. 2012;220:257-264.

73. Jensen MK, Rimm EB, Furtado JD, Sacks FM. Apolipoprotein C-III as a Potential Modulator of the Association Between HDL-Cholesterol and Incident Coronary Heart Disease. J Am Heart Assoc. 2012;1

74. Tg, Hdl Working Group of the Exome Sequencing Project NHL, Blood I, et al. Loss-of-function mutations in APOC3, triglycerides, and coronary disease. N Engl J Med. 2014;371:22-31.

75. Jorgensen AB, Frikke-Schmidt R, Nordestgaard BG, Tybjaerg-Hansen A. Loss-of-function mutations in APOC3 and risk of ischemic vascular disease. N Engl J Med. 2014;371:32-41.

76. von Eckardstein A, Holz H, Sandkamp M, et al. Apolipoprotein C-III(Lys58----Glu). Identification of an apolipoprotein C-III variant in a family with hyperalphalipoproteinemia. J Clin Invest. 1991;87:1724-1731.

77. Kawakami A, Aikawa M, Libby P, et al. Apolipoprotein CIII in apolipoprotein B lipoproteins enhances the adhesion of human monocytic cells to endothelial cells. Circulation. 2006;113:691-700.

78. Kontush A, Lhomme M, Chapman MJ. Unraveling the complexities of the HDL lipidome. J Lipid Res. 2013;54:2950-2963.

79. Papathanasiou A, Kostara C, Cung MT, et al. Analysis of the composition of plasma lipoproteins in patients with extensive coronary heart disease using 1H NMR spectroscopy. Hellenic J Cardiol. 2008;49:72-78.

80. Pruzanski W, Stefanski E, de Beer FC, et al. Comparative analysis of lipid composition of normal and acute-phase high density lipoproteins. J Lipid Res. 2000;41:1035-1047.

81. Fournier N, Francone O, Rothblat G, et al. Enhanced efflux of cholesterol from ABCA1-expressing macrophages to serum from type IV hypertriglyceridemic subjects. Atherosclerosis. 2003;171:287-293.

82. Attia N, Ramaharo A, Paul JL, et al. Enhanced removal of cholesterol from macrophage foam cells to serum from type IV hypertriglyceridemic subjects. Atherosclerosis. 2008;198:49-56.

83. Julia Z, Duchene E, Fournier N, et al. Postprandial lipemia enhances the capacity of large HDL2 particles to mediate free cholesterol efflux via SR-BI and ABCG1 pathways in type IIB hyperlipidemia. J Lipid Res. 2010;51:3350-3358.

84. Posadas-Sanchez R, Posadas-Romero C, Mendoza-Perez E, et al. Cholesterol efflux and metabolic abnormalities associated with low high-density-lipoprotein-cholesterol and high triglycerides in statin-treated coronary men with low-density lipoprotein-cholesterol <70 mg/dl. Am J Cardiol. 2012;109:636-641.

85. Greene DJ, Skeggs JW, Morton RE. Elevated triglyceride content diminishes the capacity of high density lipoprotein to deliver cholesteryl esters via the scavenger receptor class B type I (SR-BI). J Biol Chem. 2001;276:4804-4811.

86. Camont L, Lhomme M, Rached F, et al. Small, dense high-density lipoprotein-3 particles are enriched in negatively charged phospholipids: relevance to cellular cholesterol efflux, antioxidative, antithrombotic, anti-inflammatory, and antiapoptotic functionalities. Arterioscler Thromb Vasc Biol. 2013;33:2715-2723.

87. Lan Hsia S, Duncan R, Schob AH, et al. Serum levels of high-density lipoprotein phospholipids correlate inversely with severity of angiographically defined coronary artery disease. Atherosclerosis. 2000;152:469-473.

88. Piperi C, Kalofoutis C, Papaevaggeliou D, et al. The significance of serum HDL phospholipid levels in angiographically defined coronary artery disease. Clin Biochem. 2004;37:377-381.

89. Sutter I, Velagapudi S, Othman A, et al. Plasmalogens of high-density lipoproteins (HDL) are associated with coronary artery disease and anti-apoptotic activity of HDL. Atherosclerosis. 2015;241:539-546.

90. Rached F, Lhomme M, Camont L, et al. Defective functionality of small, dense HDL3 subpopulations in ST segment elevation myocardial infarction: Relevance of enrichment in lysophosphatidylcholine, phosphatidic acid and serum amyloid A. Biochim Biophys Acta. 2015;1851:1254-1261.

91. Zerrad-Saadi A, Therond P, Chantepie S, et al. HDL3-mediated inactivation of LDL-associated phospholipid hydroperoxides is determined by the redox status of apolipoprotein A-I and HDL particle surface lipid rigidity: relevance to inflammation and atherogenesis. Arterioscler Thromb Vasc Biol. 2009;29:2169-2175.

92. Yancey PG, de la Llera-Moya M, Swarnakar S, et al. High density lipoprotein phospholipid composition is a major determinant of the bi-directional flux and net movement of cellular free cholesterol mediated by scavenger receptor BI. J Biol Chem. 2000;275:36596-36604.

93. Yancey PG, Kawashiri MA, Moore R, et al. In vivo modulation of HDL phospholipid has opposing effects on SR-BI- and ABCA1-mediated cholesterol efflux. J Lipid Res. 2004;45:337-346.

94. Davidson WS, Gillotte KL, Lund-Katz S, et al. The effect of high density lipoprotein phospholipid acyl chain composition on the efflux of cellular free cholesterol. J Biol Chem. 1995;270:5882-5890.

95. Baker PW, Rye KA, Gamble JR, Vadas MA, Barter PJ. Phospholipid composition of reconstituted high density lipoproteins influences their ability to inhibit endothelial cell adhesion molecule expression. J Lipid Res. 2000;41:1261-1267.

96. Agarwala AP, Rodrigues A, Risman M, et al. High-Density Lipoprotein (HDL) Phospholipid Content and Cholesterol Efflux Capacity Are Reduced in Patients With

Very High HDL Cholesterol and Coronary Disease. Arterioscler Thromb Vasc Biol. 2015;35:1515-1519.

97. Braverman NE, Moser AB. Functions of plasmalogen lipids in health and disease. Biochim Biophys Acta. 2012;1822:1442-1452.

98. Jurgens G, Fell A, Ledinski G, Chen Q, Paltauf F. Delay of copper-catalyzed oxidation of low density lipoprotein by in vitro enrichment with choline or ethanolamine plasmalogens. Chem Phys Lipids. 1995;77:25-31.

99. Reiss D, Beyer K, Engelmann B. Delayed oxidative degradation of polyunsaturated diacyl phospholipids in the presence of plasmalogen phospholipids in vitro. Biochem J. 1997;323 (Pt 3):807-814.

100. Schwendeman A, Sviridov DO, Yuan W, et al. The effect of phospholipid composition of reconstituted HDL on its cholesterol efflux and anti-inflammatory properties. J Lipid Res. 2015;56:1727-1737.

101. Horter MJ, Sondermann S, Reinecke H, et al. Associations of HDL phospholipids and paraoxonase activity with coronary heart disease in postmenopausal women. Acta Physiol Scand. 2002;176:123-130.

102. Christoffersen C, Obinata H, Kumaraswamy SB, et al. Endothelium-protective sphingosine-1-phosphate provided by HDL-associated apolipoprotein M. Proc Natl Acad Sci U S A. 2011;108:9613-9618.

103. Sutter I, Park R, Othman A, et al. Apolipoprotein M modulates erythrocyte efflux and tubular reabsorption of sphingosine-1-phosphate. J Lipid Res. 2014;55:1730-1737.

104. Kimura T, Sato K, Kuwabara A, et al. Sphingosine 1-phosphate may be a major component of plasma lipoproteins responsible for the cytoprotective actions in human umbilical vein endothelial cells. J Biol Chem. 2001;276:31780-31785.

105. Kimura T, Sato K, Malchinkhuu E, et al. High-density lipoprotein stimulates endothelial cell migration and survival through sphingosine 1-phosphate and its receptors. Arterioscler Thromb Vasc Biol. 2003;23:1283-1288.

106. Kimura T, Tomura H, Mogi C, et al. Role of scavenger receptor class B type I and sphingosine 1-phosphate receptors in high density lipoprotein-induced inhibition of adhesion molecule expression in endothelial cells. J Biol Chem. 2006;281:37457-37467.

107. Galvani S, Sanson M, Blaho VA, et al. HDL-bound sphingosine 1-phosphate acts as a biased agonist for the endothelial cell receptor S1P1 to limit vascular inflammation. Sci Signal. 2015;8:ra79.

108. Argraves KM, Sethi AA, Gazzolo PJ, et al. S1P, dihydro-S1P and C24:1-ceramide levels in the HDL-containing fraction of serum inversely correlate with occurrence of ischemic heart disease. Lipids Health Dis. 2011;10:70.

109. Matsuo Y, Miura S, Kawamura A, et al. Newly developed reconstituted high-density lipoprotein containing sphingosine-1-phosphate induces endothelial tube formation. Atherosclerosis. 2007;194:159-168.

110. Tamama K, Tomura H, Sato K, et al. High-density lipoprotein inhibits migration of vascular smooth muscle cells through its sphingosine 1-phosphate component. Atherosclerosis. 2005;178:19-23.

111. Tao R, Hoover HE, Honbo N, et al. High-density lipoprotein determines adult mouse cardiomyocyte fate after hypoxia-reoxygenation through lipoprotein-associated sphingosine 1-phosphate. Am J Physiol Heart Circ Physiol. 2010;298:H1022-1028.

112. Brulhart-Meynet MC, Braunersreuther V, Brinck J, et al. Improving reconstituted HDL composition for efficient post-ischemic reduction of ischemia reperfusion injury. PLoS One. 2015;10:e0119664.

113. Theilmeier G, Schmidt C, Herrmann J, et al. High-density lipoproteins and their constituent, sphingosine-1-phosphate, directly protect the heart against ischemia/reperfusion injury in vivo via the S1P3 lysophospholipid receptor. Circulation. 2006;114:1403-1409.

114. Karuna R, Park R, Othman A, et al. Plasma levels of sphingosine-1-phosphate and apolipoprotein M in patients with monogenic disorders of HDL metabolism. Atherosclerosis. 2011;219:855-863.

115. Sattler KJ, Elbasan S, Keul P, et al. Sphingosine 1-phosphate levels in plasma and HDL are altered in coronary artery disease. Basic Res Cardiol. 2010;105:821-832.

116. Sattler K, Graler M, Keul P, et al. Defects of High-Density Lipoproteins in Coronary Artery Disease Caused by Low Sphingosine-1-Phosphate Content: Correction by Sphingosine-1-Phosphate-Loading. J Am Coll Cardiol. 2015;66:1470-1485.

117. Sattler K, Lehmann I, Graler M, et al. HDL-bound sphingosine 1-phosphate (S1P) predicts the severity of coronary artery atherosclerosis. Cell Physiol Biochem. 2014;34:172-184.

118. Jing XD, Wei XM, Deng SB, et al. The relationship between the high-density lipoprotein (HDL)-associated sphingosine-1-phosphate (S1P) and coronary in-stent restenosis. Clin Chim Acta. 2015;446:248-252.

119. Gomaraschi M, Ossoli A, Favari E, et al. Inflammation impairs eNOS activation by HDL in patients with acute coronary syndrome. Cardiovasc Res. 2013;100:36-43.

120. Vickers KC, Landstreet SR, Levin MG, et al. MicroRNA-223 coordinates cholesterol homeostasis. Proc Natl Acad Sci U S A. 2014;111:14518-14523.

121. Tabet F, Vickers KC, Cuesta Torres LF, et al. HDL-transferred microRNA-223 regulates ICAM-1 expression in endothelial cells. Nat Commun. 2014;5:3292.

122. Shao B, Oda MN, Oram JF, Heinecke JW. Myeloperoxidase: an oxidative pathway for generating dysfunctional high-density lipoprotein. Chem Res Toxicol. 2010;23:447-454.

123. Brennan ML, Penn MS, Van Lente F, et al. Prognostic value of myeloperoxidase in patients with chest pain. N Engl J Med. 2003;349:1595-1604.

124. Rudolph V, Keller T, Schulz A, et al. Diagnostic and prognostic performance of myeloperoxidase plasma levels compared with sensitive troponins in patients admitted with acute onset chest pain. Circ Cardiovasc Genet. 2012;5:561-568.

125. Zheng L, Nukuna B, Brennan ML, et al. Apolipoprotein A-I is a selective target for myeloperoxidase-catalyzed oxidation and functional impairment in subjects with cardiovascular disease. J Clin Invest. 2004;114:529-541.

126. Pennathur S, Bergt C, Shao B, et al. Human atherosclerotic intima and blood of patients with established coronary artery disease contain high density lipoprotein damaged by reactive nitrogen species. J Biol Chem. 2004;279:42977-42983.

127. Bergt C, Pennathur S, Fu X, et al. The myeloperoxidase product hypochlorous acid oxidizes HDL in the human artery wall and impairs ABCA1-dependent cholesterol transport. Proc Natl Acad Sci U S A. 2004;101:13032-13037.

128. Shao B, Pennathur S, Heinecke JW. Myeloperoxidase targets apolipoprotein A-I, the major high density lipoprotein protein, for site-specific oxidation in human atherosclerotic lesions. J Biol Chem. 2012;287:6375-6386.

129. Zheng L, Settle M, Brubaker G, et al. Localization of nitration and chlorination sites on apolipoprotein A-I catalyzed by myeloperoxidase in human atheroma and associated oxidative impairment in ABCA1-dependent cholesterol efflux from macrophages. J Biol Chem. 2005;280:38-47.

130. DiDonato JA, Aulak K, Huang Y, et al. Site-specific nitration of apolipoprotein A-I at tyrosine 166 is both abundant within human atherosclerotic plaque and dysfunctional. J Biol Chem. 2014;289:10276-10292.

131. Huang Y, DiDonato JA, Levison BS, et al. An abundant dysfunctional apolipoprotein A1 in human atheroma. Nat Med. 2014;20:193-203.

132. Shao B, Tang C, Heinecke JW, Oram JF. Oxidation of apolipoprotein A-I by myeloperoxidase impairs the initial interactions with ABCA1 required for signaling and cholesterol export. J Lipid Res. 2010;51:1849-1858.

133. Undurti A, Huang Y, Lupica JA, et al. Modification of high density lipoprotein by myeloperoxidase generates a pro-inflammatory particle. J Biol Chem. 2009;284:30825-30835.

134. Shao B, Oda MN, Bergt C, et al. Myeloperoxidase impairs ABCA1-dependent cholesterol efflux through methionine oxidation and site-specific tyrosine chlorination of apolipoprotein A-I. J Biol Chem. 2006;281:9001-9004.

135. Shao B, Tang C, Sinha A, et al. Humans with atherosclerosis have impaired ABCA1 cholesterol efflux and enhanced high-density lipoprotein oxidation by myeloperoxidase. Circ Res. 2014;114:1733-1742.

136. Shao B, Cavigiolio G, Brot N, Oda MN, Heinecke JW. Methionine oxidation impairs reverse cholesterol transport by apolipoprotein A-I. Proc Natl Acad Sci U S A. 2008;105:12224-12229.

137. Hewing B, Parathath S, Barrett T, et al. Effects of native and myeloperoxidase-modified apolipoprotein a-I on reverse cholesterol transport and atherosclerosis in mice. Arterioscler Thromb Vasc Biol. 2014;34:779-789.

138. Verbrugge FH, Tang WH, Hazen SL. Protein carbamylation and cardiovascular disease. Kidney Int. 2015;88:474-478.

139. Wang Z, Nicholls SJ, Rodriguez ER, et al. Protein carbamylation links inflammation, smoking, uremia and atherogenesis. Nat Med. 2007;13:1176-1184.

140. Holzer M, Gauster M, Pfeifer T, et al. Protein carbamylation renders high-density lipoprotein dysfunctional. Antioxid Redox Signal. 2011;14:2337-2346.

141. Holzer M, Zangger K, El-Gamal D, et al. Myeloperoxidase-derived chlorinating species induce protein carbamylation through decomposition of thiocyanate and urea: novel pathways generating dysfunctional high-density lipoprotein. Antioxid Redox Signal. 2012;17:1043-1052.

142. Gore MO, Luneburg N, Schwedhelm E, et al. Symmetrical dimethylarginine predicts mortality in the general population: observations from the Dallas heart study. Arterioscler Thromb Vasc Biol. 2013;33:2682-2688.

143. Siegerink B, Maas R, Vossen CY, et al. Asymmetric and symmetric dimethylarginine and risk of secondary cardiovascular disease events and mortality in patients with stable coronary heart disease: the KAROLA follow-up study. Clin Res Cardiol. 2013;102:193-202.

144. Shih DM, Gu L, Xia YR, et al. Mice lacking serum paraoxonase are susceptible to organophosphate toxicity and atherosclerosis. Nature. 1998;394:284-287.

145. Tward A, Xia YR, Wang XP, et al. Decreased atherosclerotic lesion formation in human serum paraoxonase transgenic mice. Circulation. 2002;106:484-490.

146. Huang Y, Wu Z, Riwanto M, et al. Myeloperoxidase, paraoxonase-1, and HDL form a functional ternary complex. J Clin Invest. 2013;123:3815-3828.

147. Morgantini C, Meriwether D, Baldi S, et al. HDL lipid composition is profoundly altered in patients with type 2 diabetes and atherosclerotic vascular disease. Nutr Metab Cardiovasc Dis. 2014;24:594-599.

148. Dinu AR, Merrill JT, Shen C, et al. Frequency of antibodies to the cholesterol transport protein apolipoprotein A1 in patients with SLE. Lupus. 1998;7:355-360.

149. Vuilleumier N, Reber G, James R, et al. Presence of autoantibodies to apolipoprotein A-1 in patients with acute coronary syndrome further links autoimmunity to cardiovascular disease. J Autoimmun. 2004;23:353-360.

150. Vuilleumier N, Charbonney E, Fontao L, et al. Anti-(apolipoprotein A-1) IgGs are associated with high levels of oxidized low-density lipoprotein in acute coronary syndrome. Clin Sci (Lond). 2008;115:25-33.

151. Montecucco F, Vuilleumier N, Pagano S, et al. Anti-Apolipoprotein A-1 auto-antibodies are active mediators of atherosclerotic plaque vulnerability. Eur Heart J. 2011;32:412-421.

152. Keller PF, Pagano S, Roux-Lombard P, et al. Autoantibodies against apolipoprotein A-1 and phosphorylcholine for diagnosis of non-ST-segment elevation myocardial infarction. J Intern Med. 2012;271:451-462.

153. Vuilleumier N, Bas S, Pagano S, et al. Anti-apolipoprotein A-1 IgG predicts major cardiovascular events in patients with rheumatoid arthritis. Arthritis Rheum. 2010;62:2640-2650.

154. Pagano S, Satta N, Werling D, et al. Anti-apolipoprotein A-1 IgG in patients with myocardial infarction promotes inflammation through TLR2/CD14 complex. J Intern Med. 2012;272:344-357.

155. Montecucco F, Braunersreuther V, Burger F, et al. Anti-apoA-1 auto-antibodies increase mouse atherosclerotic plaque vulnerability, myocardial necrosis and mortality triggering TLR2 and TLR4. Thromb Haemost. 2015;114:410-422.

156. Vuilleumier N, Rossier MF, Pagano S, et al. Anti-apolipoprotein A-1 IgG as an independent cardiovascular prognostic marker affecting basal heart rate in myocardial infarction. Eur Heart J. 2010;31:815-823.

157. Vuilleumier N, Montecucco F, Spinella G, et al. Serum levels of anti-apolipoprotein A-1 auto-antibodies and myeloperoxidase as predictors of major adverse cardiovascular events after carotid endarterectomy. Thromb Haemost. 2013;109:706-715.

158. Quercioli A, Montecucco F, Galan K, et al. Anti-apolipoprotein A-1 IgG levels predict coronary artery calcification in obese but otherwise healthy individuals. Mediators Inflamm. 2012;2012:243158.

159. Patel PJ, Khera AV, Jafri K, Wilensky RL, Rader DJ. The anti-oxidative capacity of high-density lipoprotein is reduced in acute coronary syndrome but not in stable coronary artery disease. J Am Coll Cardiol. 2011;58:2068-2075.

160. Ansell BJ, Navab M, Hama S, et al. Inflammatory/antiinflammatory properties of high-density lipoprotein distinguish patients from control subjects better than high-density lipoprotein cholesterol levels and are favorably affected by simvastatin treatment. Circulation. 2003;108:2751-2756.

161. Miyamoto-Sasaki M, Yasuda T, Monguchi T, et al. Pitavastatin increases HDL particles functionally preserved with cholesterol efflux capacity and antioxidative actions in dyslipidemic patients. J Atheroscler Thromb. 2013;20:708-716.

162. Guerin M, Egger P, Soudant C, et al. Dose-dependent action of atorvastatin in type IIB hyperlipidemia: preferential and progressive reduction of atherogenic apoB-containing lipoprotein subclasses (VLDL-2, IDL, small dense LDL) and stimulation of cellular cholesterol efflux. Atherosclerosis. 2002;163:287-296.

163. Niesor EJ, Schwartz GG, Perez A, et al. Statin-induced decrease in ATP-binding cassette transporter A1 expression via microRNA33 induction may counteract cholesterol efflux to high-density lipoprotein. Cardiovasc Drugs Ther. 2015;29:7-14.

164. Ginsberg HN, Reyes-Soffer G. Niacin: a long history, but a questionable future. Curr Opin Lipidol. 2013;24:475-479.

165. Yadav R, Liu Y, Kwok S, et al. Effect of Extended-Release Niacin on High-Density Lipoprotein (HDL) Functionality, Lipoprotein Metabolism, and Mediators of Vascular Inflammation in Statin-Treated Patients. J Am Heart Assoc. 2015;4

166. Green PS, Vaisar T, Pennathur S, et al. Combined statin and niacin therapy remodels the high-density lipoprotein proteome. Circulation. 2008;118:1259-1267.

167. Sorrentino SA, Besler C, Rohrer L, et al. Endothelial-vasoprotective effects of high-density lipoprotein are impaired in patients with type 2 diabetes mellitus but are improved after extended-release niacin therapy. Circulation. 2010;121:110-122.

168. Gomaraschi M, Ossoli A, Adorni MP, et al. Fenofibrate and extended-release niacin improve the endothelial protective effects of HDL in patients with metabolic syndrome. Vascul Pharmacol. 2015

169. Yvan-Charvet L, Kling J, Pagler T, et al. Cholesterol efflux potential and antiinflammatory properties of high-density lipoprotein after treatment with niacin or anacetrapib. Arterioscler Thromb Vasc Biol. 2010;30:1430-1438.

170. Franceschini G, Favari E, Calabresi L, et al. Differential effects of fenofibrate and extended-release niacin on high-density lipoprotein particle size distribution and cholesterol efflux capacity in dyslipidemic patients. J Clin Lipidol. 2013;7:414-422.

171. Khera AV, Patel PJ, Reilly MP, Rader DJ. The addition of niacin to statin therapy improves high-density lipoprotein cholesterol levels but not metrics of functionality. J Am Coll Cardiol. 2013;62:1909-1910.

172. Taylor AJ, Villines TC, Stanek EJ, et al. Extended-release niacin or ezetimibe and carotid intima-media thickness. N Engl J Med. 2009;361:2113-2122.

173. Boden WE, Probstfield JL, Anderson T, et al. Niacin in patients with low HDL cholesterol levels receiving intensive statin therapy. N Engl J Med. 2011;365:2255-2267.

174. Landray MJ, Haynes R, Hopewell JC, et al. Effects of extended-release niacin with laropiprant in high-risk patients. N Engl J Med. 2014;371:203-212.

175. Keene D, Price C, Shun-Shin MJ, Francis DP. Effect on cardiovascular risk of high density lipoprotein targeted drug treatments niacin, fibrates, and CETP inhibitors: meta-analysis of randomised controlled trials including 117,411 patients. BMJ. 2014;349:g4379.

176. Keech A, Simes RJ, Barter P, et al. Effects of long-term fenofibrate therapy on cardiovascular events in 9795 people with type 2 diabetes mellitus (the FIELD study): randomised controlled trial. Lancet. 2005;366:1849-1861.

177. Group AS, Ginsberg HN, Elam MB, et al. Effects of combination lipid therapy in type 2 diabetes mellitus. N Engl J Med. 2010;362:1563-1574.

178. Catapano AL, Farnier M, Foody JM, et al. Combination therapy in dyslipidemia: where are we now? Atherosclerosis. 2014;237:319-335.

179. Khera AV, Millar JS, Ruotolo G, Wang MD, Rader DJ. Potent peroxisome proliferator-activated receptor-alpha agonist treatment increases cholesterol efflux capacity in humans with the metabolic syndrome. Eur Heart J. 2015;36:3020-3022.

180. Triolo M, Annema W, de Boer JF, Tietge UJ, Dullaart RP. Simvastatin and bezafibrate increase cholesterol efflux in men with type 2 diabetes. Eur J Clin Invest. 2014;44:240-248.

181. Guerin M, Le Goff W, Frisdal E, et al. Action of ciprofibrate in type IIb hyperlipoproteinemia: modulation of the atherogenic lipoprotein phenotype and stimulation of high-density lipoprotein-mediated cellular cholesterol efflux. J Clin Endocrinol Metab. 2003;88:3738-3746.

182. Franceschini G, Calabresi L, Colombo C, et al. Effects of fenofibrate and simvastatin on HDL-related biomarkers in low-HDL patients. Atherosclerosis. 2007;195:385-391.

183. Fournier N, Tuloup-Minguez V, Pourci ML, et al. Fibrate treatment induced quantitative and qualitative HDL changes associated with an increase of SR-BI cholesterol efflux capacities in rabbits. Biochimie. 2013;95:1278-1287.

184. Maranghi M, Hiukka A, Badeau R, et al. Macrophage cholesterol efflux to plasma and HDL in subjects with low and high homocysteine levels: a FIELD substudy. Atherosclerosis. 2011;219:259-265.

185. Rotllan N, Llaverias G, Julve J, et al. Differential effects of gemfibrozil and fenofibrate on reverse cholesterol transport from macrophages to feces in vivo. Biochim Biophys Acta. 2011;1811:104-110.

186. Paragh G, Seres I, Harangi M, et al. The effect of micronised fenofibrate on paraoxonase activity in patients with coronary heart disease. Diabetes Metab. 2003;29:613-618.

187. Phuntuwate W, Suthisisang C, Koanantakul B, et al. Effect of fenofibrate therapy on paraoxonase1 status in patients with low HDL-C levels. Atherosclerosis. 2008;196:122-128.

188. Nissen SE, Tardif JC, Nicholls SJ, et al. Effect of torcetrapib on the progression of coronary atherosclerosis. N Engl J Med. 2007;356:1304-1316.

189. Nicholls SJ, Tuzcu EM, Brennan DM, Tardif JC, Nissen SE. Cholesteryl ester transfer protein inhibition, high-density lipoprotein raising, and progression of coronary atherosclerosis: insights from ILLUSTRATE (Investigation of Lipid Level Management Using Coronary Ultrasound to Assess Reduction of Atherosclerosis by CETP Inhibition and HDL Elevation). Circulation. 2008;118:2506-2514.

190. Barter PJ, Caulfield M, Eriksson M, et al. Effects of torcetrapib in patients at high risk for coronary events. N Engl J Med. 2007;357:2109-2122.

191. Cannon CP, Shah S, Dansky HM, et al. Safety of anacetrapib in patients with or at high risk for coronary heart disease. N Engl J Med. 2010;363:2406-2415.

192. Brinton EA, Kher U, Shah S, et al. Effects of anacetrapib on plasma lipids in specific patient subgroups in the DEFINE (Determining the Efficacy and Tolerability of CETP INhibition with AnacEtrapib) trial. J Clin Lipidol. 2015;9:65-71.

193. Curb JD, Abbott RD, Rodriguez BL, et al. A prospective study of HDL-C and cholesteryl ester transfer protein gene mutations and the risk of coronary heart disease in the elderly. J Lipid Res. 2004;45:948-953.

194. Koropatnick TA, Kimbell J, Chen R, et al. A prospective study of high-density lipoprotein cholesterol, cholesteryl ester transfer protein gene variants, and healthy aging in very old Japanese-american men. J Gerontol A Biol Sci Med Sci. 2008;63:1235-1240.

195. Barzilai N, Atzmon G, Schechter C, et al. Unique lipoprotein phenotype and genotype associated with exceptional longevity. JAMA. 2003;290:2030-2040.

196. Soerensen M, Dato S, Tan Q, et al. Evidence from case-control and longitudinal studies supports associations of genetic variation in APOE, CETP, and IL6 with human longevity. Age (Dordr). 2013;35:487-500.

197. Zhong S, Sharp DS, Grove JS, et al. Increased coronary heart disease in Japanese-American men with mutation in the cholesteryl ester transfer protein gene despite increased HDL levels. J Clin Invest. 1996;97:2917-2923.

198. Hirano K, Yamashita S, Kuga Y, et al. Atherosclerotic disease in marked hyperalphalipoproteinemia. Combined reduction of cholesteryl ester transfer protein and hepatic triglyceride lipase. Arterioscler Thromb Vasc Biol. 1995;15:1849-1856.

199. Thompson A, Di Angelantonio E, Sarwar N, et al. Association of cholesteryl ester transfer protein genotypes with CETP mass and activity, lipid levels, and coronary risk. JAMA. 2008;299:2777-2788.

200. Kathiresan S. Will cholesteryl ester transfer protein inhibition succeed primarily by lowering low-density lipoprotein cholesterol? Insights from human genetics and clinical trials. J Am Coll Cardiol. 2012;60:2049-2052.

201. Wu Z, Lou Y, Qiu X, et al. Association of cholesteryl ester transfer protein (CETP) gene polymorphism, high density lipoprotein cholesterol and risk of coronary artery disease: a meta-analysis using a Mendelian randomization approach. BMC Med Genet. 2014;15:118.

202. Ritsch A, Scharnagl H, Eller P, et al. Cholesteryl ester transfer protein and mortality in patients undergoing coronary angiography: the Ludwigshafen Risk and Cardiovascular Health study. Circulation. 2010;121:366-374.

203. Khera AV, Wolfe ML, Cannon CP, Qin J, Rader DJ. On-statin cholesteryl ester transfer protein mass and risk of recurrent coronary events (from the pravastatin or atorvastatin evaluation and infection therapy-thrombolysis in myocardial infarction 22 [PROVE IT-TIMI 22] study). Am J Cardiol. 2010;106:451-456.

204. de Vries-van der Weij J, Zadelaar S, Toet K, et al. Human CETP aggravates atherosclerosis by increasing VLDL-cholesterol rather than by decreasing HDL-cholesterol in APOE*3-Leiden mice. Atherosclerosis. 2009;206:153-158.

205. Plump AS, Masucci-Magoulas L, Bruce C, et al. Increased atherosclerosis in ApoE and LDL receptor gene knock-out mice as a result of human cholesteryl ester transfer protein transgene expression. Arterioscler Thromb Vasc Biol. 1999;19:1105-1110.

206. Rittershaus CW, Miller DP, Thomas LJ, et al. Vaccine-induced antibodies inhibit CETP activity in vivo and reduce aortic lesions in a rabbit model of atherosclerosis. Arterioscler Thromb Vasc Biol. 2000;20:2106-2112.

207. Okamoto H, Yonemori F, Wakitani K, et al. A cholesteryl ester transfer protein inhibitor attenuates atherosclerosis in rabbits. Nature. 2000;406:203-207.

208. Morehouse LA, Sugarman ED, Bourassa PA, et al. Inhibition of CETP activity by torcetrapib reduces susceptibility to diet-induced atherosclerosis in New Zealand White rabbits. J Lipid Res. 2007;48:1263-1272.

209. Sugano M, Makino N, Sawada S, et al. Effect of antisense oligonucleotides against cholesteryl ester transfer protein on the development of atherosclerosis in cholesterol-fed rabbits. J Biol Chem. 1998;273:5033-5036.

210. Yvan-Charvet L, Matsuura F, Wang N, et al. Inhibition of cholesteryl ester transfer protein by torcetrapib modestly increases macrophage cholesterol efflux to HDL. Arterioscler Thromb Vasc Biol. 2007;27:1132-1138.

211. Bellanger N, Julia Z, Villard EF, et al. Functionality of postprandial larger HDL2 particles is enhanced following CETP inhibition therapy. Atherosclerosis. 2012;221:160-168.

212. Catalano G, Julia Z, Frisdal E, et al. Torcetrapib differentially modulates the biological activities of HDL2 and HDL3 particles in the reverse cholesterol transport pathway. Arterioscler Thromb Vasc Biol. 2009;29:268-275.

213. Tchoua U, D'Souza W, Mukhamedova N, et al. The effect of cholesteryl ester transfer protein overexpression and inhibition on reverse cholesterol transport. Cardiovasc Res. 2008;77:732-739.

214. Niesor EJ, Magg C, Ogawa N, et al. Modulating cholesteryl ester transfer protein activity maintains efficient pre-beta-HDL formation and increases reverse cholesterol transport. J Lipid Res. 2010;51:3443-3454.

215. Ballantyne CM, Miller M, Niesor EJ, et al. Effect of dalcetrapib plus pravastatin on lipoprotein metabolism and high-density lipoprotein composition and function in dyslipidemic patients: results of a phase IIb dose-ranging study. Am Heart J. 2012;163:515-521, 521 e511-513.

216. Ray KK, Ditmarsch M, Kallend D, et al. The effect of cholesteryl ester transfer protein inhibition on lipids, lipoproteins, and markers of HDL function after an acute coronary syndrome: the dal-ACUTE randomized trial. Eur Heart J. 2014;35:1792-1800.

217. Briand F, Thieblemont Q, Muzotte E, et al. Anacetrapib and dalcetrapib differentially alters HDL metabolism and macrophage-to-feces reverse cholesterol transport at similar levels of CETP inhibition in hamsters. Eur J Pharmacol. 2014;740:135-143.

218. Riwanto M, Deanfield J, Manz J, et al. Vascular Effects of High-Density Lipoprotein after CETP Inhibition with Dalcetrapib in Patients with Stable Coronary Disease or an Acute Coronary Syndrome. AHA Sessions. 2012

219. Tardif JC, Rheaume E, Lemieux Perreault LP, et al. Pharmacogenomic determinants of the cardiovascular effects of dalcetrapib. Circ Cardiovasc Genet. 2015;8:372-382.

220. Castro-Perez J, Briand F, Gagen K, et al. Anacetrapib promotes reverse cholesterol transport and bulk cholesterol excretion in Syrian golden hamsters. J Lipid Res. 2011;52:1965-1973.

221. Han S, Levoci L, Fischer P, et al. Inhibition of cholesteryl ester transfer protein by anacetrapib does not impair the anti-inflammatory properties of high density lipoprotein. Biochim Biophys Acta. 2013;1831:825-833.

222. Uehara Y, Chiesa G, Saku K. High-Density Lipoprotein-Targeted Therapy and Apolipoprotein A-I Mimetic Peptides. Circ J. 2015;79:2523-2528.

223. Stoekenbroek RM, Stroes ES, Hovingh GK. ApoA-I mimetics. Handb Exp Pharmacol. 2015;224:631-648.

224. Tardif JC, Gregoire J, L'Allier PL, et al. Effects of reconstituted high-density lipoprotein infusions on coronary atherosclerosis: a randomized controlled trial. JAMA. 2007;297:1675-1682.

225. Spieker LE, Sudano I, Hurlimann D, et al. High-density lipoprotein restores endothelial function in hypercholesterolemic men. Circulation. 2002;105:1399-1402.

226. Bisoendial RJ, Hovingh GK, Levels JH, et al. Restoration of endothelial function by increasing high-density lipoprotein in subjects with isolated low high-density lipoprotein. Circulation. 2003;107:2944-2948.

227. Drew BG, Duffy SJ, Formosa MF, et al. High-density lipoprotein modulates glucose metabolism in patients with type 2 diabetes mellitus. Circulation. 2009;119:2103-2111.

228. Patel S, Drew BG, Nakhla S, et al. Reconstituted high-density lipoprotein increases plasma high-density lipoprotein anti-inflammatory properties and cholesterol efflux capacity in patients with type 2 diabetes. J Am Coll Cardiol. 2009;53:962-971.

229. Hoang A, Drew BG, Low H, et al. Mechanism of cholesterol efflux in humans after infusion of reconstituted high-density lipoprotein. Eur Heart J. 2012;33:657-665.

230. Huang Y, von Eckardstein A, Assmann G. Cell-derived unesterified cholesterol cycles between different HDLs and LDL for its effective esterification in plasma. Arterioscler Thromb. 1993;13:445-458.

231. Gille A, Easton R, D'Andrea D, Wright SD, Shear CL. CSL112 enhances biomarkers of reverse cholesterol transport after single and multiple infusions in healthy subjects. Arterioscler Thromb Vasc Biol. 2014;34:2106-2114.

232. Ibanez B, Giannarelli C, Cimmino G, et al. Recombinant HDL(Milano) exerts greater anti-inflammatory and plaque stabilizing properties than HDL(wild-type). Atherosclerosis. 2012;220:72-77.

233. Nissen SE, Tsunoda T, Tuzcu EM, et al. Effect of recombinant ApoA-I Milano on coronary atherosclerosis in patients with acute coronary syndromes: a randomized controlled trial. JAMA. 2003;290:2292-2300.

234. Kempen HJ, Gomaraschi M, Bellibas SE, et al. Effect of repeated apoA-IMilano/POPC infusion on lipids, (apo)lipoproteins, and serum cholesterol efflux capacity in cynomolgus monkeys. J Lipid Res. 2013;54:2341-2353.

235. Kempen HJ, Schranz DB, Asztalos BF, et al. Incubation of MDCO-216 (ApoA-IMilano/POPC) with Human Serum Potentiates ABCA1-Mediated Cholesterol Efflux Capacity, Generates New Prebeta-1 HDL, and Causes an Increase in HDL Size. J Lipids. 2014;2014:923903.

236. Barbaras R. Non-clinical development of CER-001. Front Pharmacol. 2015;6:220.

237. Kootte RS, Smits LP, van der Valk FM, et al. Effect of open-label infusion of an apoA-I-containing particle (CER-001) on RCT and artery wall thickness in patients with FHA. J Lipid Res. 2015;56:703-712.

238. Hovingh GK, Smits LP, Stefanutti C, et al. The effect of an apolipoprotein A-I-containing high-density lipoprotein-mimetic particle (CER-001) on carotid artery wall thickness in patients with homozygous familial hypercholesterolemia: The Modifying Orphan Disease Evaluation (MODE) study. Am Heart J. 2015;169:736-742 e731.

239. Tardif JC, Ballantyne CM, Barter P, et al. Effects of the high-density lipoprotein mimetic agent CER-001 on coronary atherosclerosis in patients with acute coronary syndromes: a randomized trial. Eur Heart J. 2014;35:3277-3286.

240. O'Neill F, McLoughlin E, Riwanto M, et al. Reproducibility and biological variability of HDL's vascular functional assays. Atherosclerosis. 2015;241:588-594.

241. Morrow DA, de Lemos JA. Benchmarks for the assessment of novel cardiovascular biomarkers. Circulation. 2007;115:949-952.

242. Vasan RS. Biomarkers of cardiovascular disease: molecular basis and practical considerations. Circulation. 2006;113:2335-2362.

243. Yamashita S, Masuda D, Matsuzawa Y. Did we abandon probucol too soon? Curr Opin Lipidol. 2015;26:304-316.

244. Wu FC, von Eckardstein A. Androgens and coronary artery disease. Endocr Rev. 2003;24:183-217.

Table 1: Effects of compositional changes in high-density lipoproteins (HDL) on HDL function

Modification	Functional impact	Potential mechanism	References
SAA-enrichment	↓ Cholesterol efflux capacity ↓ Macrophage reverse cholesterol transport ↓ Inhibition of MCP-1 Disturbed endothelial function: - No inhibition of VCAM-1 expression - No inhibition of monocyte adhesion to ECs - No stimulation of NO production ↓ Anti-oxidative activity	Proteoglycan retention ↓ Cholesterol efflux and SR-BI-mediated selective uptake of HDL-CE ↑ FPR2-mediated signaling ↑ MCP-1 and VCAM-1 expression ↓ PON1 and PAF-AH activity	60, 61, 68 62, 63 64 65 67
ApoC-III-enrichment	Disturbed endothelial function: - No inhibition of monocyte adhesion to ECs - ↓ Inhibition of endothelial apoptosis	 ↑ Phosphorylation of p38-MAPK and proapoptotic tBid expression	 77 21
Triglyceride-enrichment	↓ Cholesterol efflux capacity ↓ SR-BI-mediated selective uptake of HDL-CE ↓ Inhibition of IL-8 release from ECs	Competition of HDL-TG with HDL-CE for selective uptake by SR-BI	47 83, 85 47
Sphingomyelin-depletion	↓ Anti-oxidative activity ↓ Cholesterol efflux capacity ↓ Inhibition of cytokine release	↑ HDL surface lipid rigidity	91 100 100
Phosphatidylserine-enrichment	↑ Cholesterol efflux capacity ↑ Inhibition of LDL oxidation ↑ Inhibition of platelet activation ↑ Anti-inflammatory activity ↑ Inhibition of endothelial apoptosis	↑ Negative HDL surface charge	86
Phosphatidylcholine-enrichment	↑ Cholesterol efflux capacity	↑ Solubilization of desorbed cholesterol molecules	86, 92
Lysophosphatidylcholine-enrichment	↓ Cholesterol efflux capacity ↓ Inhibition of LDL oxidation	Dissociation of apoA-I from HDL	90
Phosphatidic acid-enrichment	↓ Cholesterol efflux capacity		90
Plasmalogen PC35:2-enrichment	↑ Inhibition of endothelial apoptosis	Scavenging of oxygen radicals, reduction of cholesterol oxidation by free radicals, or delayed oxidation of polyunsaturated fatty acids	89
S1P-depletion	Disturbed endothelial function: - ↓ eNOS activation and stimulation of NO production - ↓ Vasodilator potency	↓ ERK1/2 and Akt signaling ↓ NO production	116, 119 116

	- ↓ Inhibition of endothelial apoptosis - ↓ Endothelial barrier function		[89] [108]
SDMA-enrichment	Disturbed endothelial function: - Inhibition of NO production - ↓ Stimulation of EC migration - ↓ Stimulation of endothelial repair - ↑ VCAM-1 expression - ↑ Superoxide production ↑ Blood pressure in mice	Activation of endothelial TLR2-signaling: ↓ eNOS-activating phosphorylation, ↑ eNOS-inhibiting phosphorylation Activation of NADPH oxidase	[41]
MDA-enrichment	Disturbed endothelial function: - ↓ Stimulation of NO production	Activation of LOX-1 and protein kinase C β3	[40]

Abbreviations used: Apo = apolipoprotein; CE = cholesteryl ester; EC = endothelial cell; eNOS = endothelial nitric oxide synthase; FPR2 = formyl-peptide receptor 2; IL-8= interleukin-8; LDL = low-density lipoprotein; LOX-1 = lectin-like oxidized LDL receptor; MCP-1 = monocyte chemotactic protein-1; MDA = malondialdehyde; NO = nitric oxide; PON1 = paraoxonase 1; PAF-AH = platelet activating factor acetylhydrolase; S1P = sphingosine-1-phosphate; SAA = serum amyloid A; SDMA = symmetric dimethylarginine; SR-BI = scavenger receptor class B type I; TG = triglyceride; TLR2 = toll-like receptor 2; VCAM-1 = vascular cell adhesion molecule 1

Table 2: Major amino acid residues in apolipoprotein (apo)A-I modified by myeloperoxidase (MPO) and effects on HDL function

Amino acid residue in apoA-I	Preferred modification	Functional impact	Proportion of apoA-I molecules modified	Causal role in apoA-I dysfunction
Tyr-192	Chlorination	↓ Cholesterol efflux capacity	Plasma: <0.02% Plaque: 20%	Unclear
Tyr-166	Nitration	= Cholesterol efflux capacity ↓ Stimulation of LCAT activity	Plasma: 0.14% Plaque: 8%	Unclear
Trp-72	Oxidation	↓ Cholesterol efflux capacity ↓ Stimulation of LCAT activity Loss of HDL biogenesis activity *in vivo* ↑ VCAM-1 protein expression on ECs ↑ Nuclear translocation of NF-κB in ECs	Plasma: 0.007% Plaque: 20%	Yes
Met-148	Sulfoxidation	↓ Cholesterol efflux capacity ↓ Stimulation of LCAT activity	Plasma: 30% Plaque: ?	Unclear

Abbreviations used: EC = endothelial cell; HDL = high-density lipoprotein; LCAT = lecithin-cholesterol acyltransferase; Met = methionine; Tyr = tyrosine; Trp = tryptophan; VCAM-1 = vascular cell adhesion molecule 1

Table 3: Effects of lipid modifying drugs on high-density lipoprotein (HDL) cholesterol, HDL function and cardiovascular disease

	statins	fibrates	niacin	CETP inhibitors	HDL mimetics
HDL cholesterol level	No or little effect	Increase by 5 to 15%	Increase by 10 to 35%	Increase by 25% (dalcetrapib) to 130% (anacetrapib)	No persistent increase
Cholesterol efflux capacity	No consistent effect	Increased ABCA1-dependent efflux	No effect independently of HDL cholesterol	Increased ABCA1-dependent and ABCA1-independent efflux	increased ABCA1-dependent and ABCA1-independent efflux
Anti-oxidant activities	Possibly increased PON1 activity	No effect on PON1	No effect on PON1	?	?
Endothelial functionality	?	Increased stimulation of eNOS and inhibition of VCAM-1	Increased stimulation of eNOS and inhibition of VCAM-1	No change in eNOS stimulation or inhibition of VCAM-1 or apoptosis	Increased inhibition of VCAM-1 by CSL111
Extent of atherosclerosis	Reduced progression. Regression upon Intensive treatment	Reduced progression	Reduced progression.	No change by torcetrapib or dalcetrapib. Evacetrapib and anacytrapib unkonwn	Controversial evidence
Cardiovascular endpoints	Reduction of cardiovascular morbidity and mortality as well as total mortality	Reduced cardiovascular morbidity in the absence of statins; no effect if combined with statins	Reduced cardiovascular mortality in the absence of statins; no effect if combined with statins	Increased cardiovascular morbidity and total mortality by torcetrapib, lack of effect by dalcetrapib or evacetrapib, anacetrapib unknown	Not known

Abbreviations used: ABCA1 = ATP-binding cassette transport A1; eNOS = endothelial nitric oxide synthase; PON1 = paraoxonase 1; VCAM-1 = vascular cell adhesion molecule 1.

Table 4: Rating of high-density lipoprotein (HDL)-related biomarkers according to clinical utility criteria[241, 242]

	HDL cholesterol	apoA-I	HDL particle number	HDL subclasses	Cholesterol efflux capacity	Anti-oxidative capacity (PON1)	Endothelial function
Analytical performance							
Precise assays	Yes	Yes	(yes)	(yes)	yes	yes	Yes
Accurate/method-independent assays?	Not optimal	Yes	(yes)	No	no	PON1 yes	Unknown
Pre-analytical issues clarified?	Yes	Yes	(yes)	(yes)	(yes)	(yes)	(yes)
Accessible assays?	Yes	Yes	no	No	no	No (PON1 activity feasible)	no
High throughput and rapid turn-around-time?	Yes	Yes	Within a center yes, otherwise no	Within a center by NMR yes, otherwise no	no	No (PON1 activity feasible)	No
Reasonable costs?	Yes	Yes	?	?	no	? (PON1 activity feasible)	no
Diagnostic / prognostic performance							
Robust association with incident disease?	Yes	Yes	yes	No	(Yes)	PON1 No, otherwise unknown	Unknown
Novel information beyond existing biomarkers?	(reference)	No	(yes)	No	In positive studies yes	PON1 No, otherwise unknown	Unknown
Validated decision limits?	Yes	No	no	No	No	No	no
Clinical utility							
Superiority to existing tests?	(Reference)	No	Possibly	Unknown	Unknown	Unknown	Unknown
Modifiable risk association (treatment target)?	No	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown
Biomarker guided triage enhances care?	yes	unknown	unknown	Unknown	unknown	unknown	unknown

Abbreviations used: apo = apolipoprotein; PON1 = paraoxonase 1